Selective Estrogen Receptor Modulatory Effects of Soy Isoflavones: Mechanistic Insights and Clinical Applications Across the Neuro–Endocrine–Metabolic Axes

From the Estrogen Receptor-β and Gut–Hormone Interaction Axis to Systemic Hormonal Homeostasis – An Evidence-Based Nutritional Pharmacology Review in Menopausal Syndrome, Osteopenia, Premenstrual Syndrome (PMS), and Metabolic Dysregulation

Abstract

Background:

Postmenopausal and hormone-disordered states such as polycystic ovary syndrome (PCOS) and estrogen-dominant conditions share a common pathophysiological foundation of hypothalamic–pituitary–ovarian (HPO) feedback disruption, chronic inflammation, and metabolic dysregulation.

Soy isoflavones, as selective estrogen receptor-β (ER-β) agonists, exhibit receptor-specific and system-level modulatory properties distinct from conventional estrogen replacement therapy.

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Objective:

To elucidate the bidirectional and system-integrative regulatory mechanisms of soy isoflavones on the neuro-endocrine–metabolic tri-axis, and to define their synergistic interactions with complementary nutrients within the Keyora nutritional pharmacology framework.

Methods and Mechanistic Insights:

Soy isoflavones (genistein, daidzein) selectively activate ER-β in neural, endocrine, and metabolic tissues, rebalancing the ER-α/ER-β ratio and restoring feedback sensitivity of HPO and HPA axes. This action suppresses excessive GnRH/LH pulsing, normalizes FSH responsiveness, and stabilizes ovarian steroidogenesis.

At the cellular level, ER-β activation coordinates PI3K–AKT–AMPK–PGC1α energy signaling and Nrf2–NF-κB redox balance, reducing oxidative and inflammatory stress in ovarian, vascular, and hepatic tissues. Through these mechanisms, soy isoflavones simultaneously enhance RANKL/OPG-mediated bone remodeling, eNOS–NO–PGC1α endothelial function, and insulin sensitivity.

Synergistic Nutrient Network:

Within the Keyora multi-nutrient model, soy isoflavones operate as a signaling core amplified by co-factors - Vitex agnus-castus (dopamine D_2–PRL axis), magnesium (AMPK activation), selenium and vitamin E (GPx/Nrf2 antioxidant defense), and vitamin

B6 (CYP–COMT-dependent estrogen metabolism).

Together they form a four-dimensional feedback network integrating neural, endocrine, metabolic, and oxidative axes, achieving system-wide homeostatic reinstatement rather than symptom-specific correction.

Results and Clinical Implications:

Evidence from randomized controlled trials and meta-analyses demonstrates that soy isoflavone supplementation (40-80 mg/day) improves bone mineral density, vascular endothelial function, insulin sensitivity, and ovulatory rhythm, while reducing inflammatory markers (CRP, IL-6, TNF-α) and vasomotor or mood symptoms. These outcomes validate the concept of signal-driven homeostatic reconstruction, with ER-β serving as the central convergence node across bone–vascular–metabolic systems.

Conclusion:

Soy isoflavones represent a paradigm shift from hormonal substitution to selective receptor and systems-level recalibration. Acting as physiological signal modulators, they restore equilibrium across the neuro-endocrine–metabolic network through receptor selectivity, energy re-synchronization, and anti-inflammatory coherence.

In combination with Keyora’s synergistic nutrient design, they embody a nutripharmacological strategy for precision management of PCOS, estrogen-dominant disorders, and postmenopausal metabolic decline.

Keywords:

Soy Isoflavones； Estrogen Receptor beta；Hypothalamic–Pituitary–Ovarian Axis；Polycystic Ovary Syndrome；Estrogen Dominance pathology；Signal Transduction；AMP-Activated Protein Kinases；Nuclear Factor Erythroid 2–Related Factor 2；NF-κB / antagonists；Mitochondria；Oxidative Stress / drug effects；Inflammation；Bone Remodeling；Vascular Endothelium；Insulin Resistance；Neuroendocrine System.

Soy isoflavones are a class of naturally occurring polyphenolic compounds present in soybeans (Glycine max) and soy-derived foods. They belong to the flavonoid family and constitute a major subgroup of phytoestrogens. Owing to their structural similarity to 17β-estradiol, the primary endogenous estrogen in humans, soy isoflavones can bind to estrogen receptors (ERs) and exert either estrogen-like or anti-estrogenic physiological effects depending on the hormonal milieu.

The principal bioactive components of soy isoflavones are genistein, daidzein, and glycitein, with genistein and daidzein being the most abundant and biologically potent. In their natural state, these compounds predominantly exist as glycosides (genistin, daidzin, glycitin), which must be hydrolyzed by intestinal β-glucosidases to their aglycone (free) forms before absorption and biological activation can occur.

Initially classified as “weak plant estrogens,” soy isoflavones are now recognized to act in a manner more akin to Selective Estrogen Receptor Modulators (SERM-like) rather than simple hormone mimetics. Their binding affinities differ significantly between the two estrogen receptor subtypes:

- High affinity for ER-β (estrogen receptor-β)
- Low affinity for ER-α

This receptor selectivity underlies their bidirectional regulatory nature - in hypo-estrogenic conditions such as perimenopause or menopause, they provide mild receptor activation to compensate for hormonal decline; conversely, in hyper-estrogenic states, they exhibit partial antagonism to prevent excessive receptor stimulation, thereby supporting hormonal homeostasis. Notably, equol, a daidzein-derived metabolite produced by specific intestinal bacteria, exhibits even stronger ER-β selectivity and superior antioxidant capacity, serving as a key biomarker of individual responsiveness to soy isoflavone intake.

After oral ingestion, soy isoflavones are hydrolyzed and absorbed mainly in the small intestine in aglycone form, while some glycosides undergo further microbial metabolism in the colon. Absorbed isoflavones are conjugated via glucuronidation and sulfation in the liver and subsequently excreted in the urine. Their bioavailability is influenced by multiple factors including gut microbiota composition, food matrix, lipid environment, and genetic polymorphisms of metabolic enzymes. Epidemiological evidence suggests that the prevalence of “equol producers” is higher among Asian populations, which may partly explain the greater cardio-metabolic, skeletal, and menopausal benefits observed in populations adhering to soy-rich dietary patterns.

From an integrative nutritional-pharmacological standpoint, soy isoflavones can be conceptualized as regulatory agents across the neuro–endocrine–metabolic axes:

- Neuro-endocrine axis: Modulate neurotransmission and vascular tone through ER-β and membrane-bound GPER1 signaling pathways.
- Metabolic–skeletal axis: Regulate the RANKL/OPG balance, promoting osteogenesis while suppressing osteoclast activity.
- Inflammatory–oxidative axis: Inhibit NF-κB activation, enhance Nrf2–ARE signaling, and mitigate oxidative stress and inflammatory damage.

Thus, soy isoflavones function not merely as auxiliary hormonal nutrients but as systemic modulators influencing sleep, mood, bone density, endothelial function, and overall metabolic health. Within typical physiological intake ranges (expressed as aglycone equivalents, 40-100 mg/day), soy isoflavones demonstrate an excellent safety profile. Randomized controlled trials have consistently shown significant or moderate efficacy in alleviating menopausal hot flashes, improving sleep quality, enhancing bone mineral density, and optimizing lipid metabolism.

Long-term safety is further supported by epidemiological data, although individuals with a history of estrogen-dependent tumors should be evaluated carefully and use such supplements under medical supervision when necessary.

I Major Active Components and Core Mechanistic Pathways of Soy Isoflavones

Throughout a woman's life course - from the cyclical hormonal fluctuations of the menstrual period to the endocrine decline of menopause - imbalanced estrogen signaling is a central factor underlying a spectrum of neuroendocrine and metabolic disturbances. Symptoms such as hot flashes, night sweats, anxiety, sleep disruption, menstrual irregularities, and bone density loss are all closely associated with dysregulation of estrogen receptor (ER) activity.

In traditional pharmacology, exogenous hormone replacement therapy (HRT) can alleviate these symptoms in the short term; however, long-term use carries increased risks related to breast and endometrial tissue proliferation. Consequently, the search for naturally derived estrogen modulators with both receptor selectivity and physiological safety has become a key focus in nutritional pharmacology.

Against this backdrop, soy isoflavones have drawn extensive scientific attention due to their unique combination of structural similarity to estrogens and functional selectivity in receptor interactions. They not only mimic certain physiological actions of endogenous estrogens under low-estrogen conditions but also exert antagonistic effects in hyper-estrogenic environments - thereby maintaining dynamic hormonal equilibrium. Their core mechanism is built upon a three-dimensional regulatory network that integrates ER-β–selective activation, GPER1-mediated rapid signaling, and gut–hormone feedback loops.

Importantly, soy isoflavones are not a single active compound but rather a polyphenolic complex with structural complementarity. The three principal constituents - genistein, daidzein, and glycitein - possess distinct receptor affinities and antioxidant potentials, acting synergistically to stabilize the neuro–endocrine–metabolic tri-axis.

Moreover, the intestinal metabolite equol represents a critical amplification node within this network. By markedly enhancing ER-β activation and antioxidant capacity, equol establishes a closed-loop interaction among the host, gut microbiota, and hormonal system - a functional characteristic absent in *Vitex agnus-castus*.

Thus, research on soy isoflavones has evolved beyond the conventional concept of “plant estrogens.” It now represents a nutritional pharmacology model centered on receptor selectivity, gut-derived synergy, and systemic homeostatic reconstruction.

The Keyora framework will systematically elucidate their principal bioactive components, receptor-binding characteristics, signal transduction pathways, and gut–hormone interaction mechanisms - laying the physiological and molecular groundwork for subsequent discussions on the integrative neuro–endocrine–metabolic tri-axis and symptom-directed clinical applications.

1) Composition and Structural Characteristics of the Active Constituents

Soy isoflavones are a class of well-characterized polyphenolic flavonoids, representing one of the most extensively studied families of phytoestrogens to date. Their core chemical scaffold is the isoflavone nucleus (C_6–C_3–C_6), which differs structurally from classical flavonoids in that the aromatic B-ring is attached to the C-ring at the C-3 position rather than C-2.

This positional shift gives rise to a unique planar configuration that closely resembles the spatial conformation and electron density distribution of 17β-estradiol (E2), enabling direct binding to estrogen receptors and subsequent activation of estrogen-responsive signaling pathways.

1.1) The Three Principal Monomeric Compounds

The major active constituents of soy isoflavones comprise three structurally related yet functionally complementary monomers:

- Genistein: A tri-hydroxylated isoflavone (5,7,4′-trihydroxyisoflavone) and the most potent bioactive molecule of the group. Its highly polar hydroxyl clusters form a stable hydrogen-bonding network within the ER-β ligand-binding pocket, conferring the strongest estrogenic activity among the isoflavones. In addition, genistein exhibits significant antioxidant and tyrosine kinase–inhibitory properties.
- Daidzein: A structural analogue of genistein with comparatively weaker ER-β affinity. However, it serves as a precursor to equol, a high-potency intestinal metabolite produced by specific bacterial strains, which possesses greater biological activity and a longer plasma half-life.
- Glycitein: Characterized by a methoxy substituent, glycitein has lower receptor affinity but contributes auxiliary benefits through its anti-glycation and free radical–scavenging capacities.

Collectively, these three monomers constitute a “structural synergy cluster”, functioning as a multi-targeted, temporally regulated hormonal modulation system in vivo.

1.2) Transformation Between Glycoside and Aglycone Forms

In nature, soy isoflavones predominantly exist as glycoside conjugates - namely, genistin, daidzin, and glycitin. Within the upper small intestine, these conjugates are hydrolyzed by β-glucosidases, cleaving the glycosidic bond to yield the aglycone form, which is the absorbable and bioactive state.

This glycoside-to-aglycone conversion determines both absorption efficiency and pharmacokinetic profile. Clinical investigations have shown that plasma concentrations of aglycone isoflavones are two- to threefold higher, and their time-to-peak levels occur one to two hours earlier compared with their glycoside counterparts. Thus, enzymatic hydrolysis is a crucial step governing the bioavailability of soy isoflavones.

1.3) Structure–Function Correlation

The biological activity of soy isoflavones is tightly governed by their substitution patterns and functional groups:

- The presence of 4′-hydroxyl and 7-hydroxyl groups is critical for ER-β binding.
- The 5-hydroxyl group promotes π–π stacking interactions with aromatic residues in the receptor, enhancing binding stability.
- Methoxy substitution modulates lipophilicity and membrane permeability, influencing cellular distribution and the intensity of non-genomic signaling.

These substituent configurations define the hierarchy of receptor affinity among the compounds:

Genistein > Equol > Daidzein > Glycitein,

which also underpins their differential potency in ER-β–selective activation and GPER1-mediated rapid signaling pathways.

1.4) Systemic Correlation with Physiological Functions

From a nutritional pharmacology perspective, the structural framework of soy isoflavones endows them with three principal physiological properties:

- SERM-like activity – Sustaining receptor-mediated estrogenic signaling under low-estrogen conditions through selective activation of ER-β.
- Antioxidant and anti-inflammatory actions – Phenolic hydroxyl groups confer potent radical-scavenging ability and inhibition of the NF-κB inflammatory cascade.
- Signal amplification and synergistic potential – Structural complementarity with the gut-derived metabolite equol magnifies regulatory effects across bone, endothelial, and metabolic axes.

Therefore, from a chemical standpoint, soy isoflavones represent not merely prototypical phytoestrogens but a structurally selective, multi-target molecular ensemble capable of orchestrating systemic endocrine homeostasis. This structure-driven functional complexity provides the material foundation for subsequent mechanistic analyses within the neuro–endocrine–metabolic tri-axis framework.

2) Selective Estrogen Receptor Binding and Signaling Characteristics

2.1) Receptor Subtype Differentiation and Selective Binding Mechanism

Estrogen receptors (ERs) comprise two major nuclear receptor subtypes, ER-α and ER-β, which, despite structural homology, differ significantly in tissue distribution and functional regulation:

- ER-α is predominantly expressed in the breast, endometrium, and liver, where it mediates reproductive and anabolic processes.
- ER-β, by contrast, is abundant in the brain, skeletal tissue, vascular endothelium, immune system, and intestinal tract, where it plays key roles in neuroprotection, bone metabolism, cardiovascular homeostasis, and emotional regulation.

The structural characteristics of soy isoflavones confer markedly higher binding affinity to ER-β than to ER-α, with an affinity ratio estimated between 20:1 and 80:1. This selectivity arises because the ligand-binding pocket of ER-β is smaller and more polar, favoring the polyhydroxylated and planar aromatic configuration of isoflavones.

Consequently, soy isoflavones exhibit a “ER-β–selective activation and ER-α–weak binding” profile, providing mild receptor activation under hypo-estrogenic conditions while avoiding overstimulation of ER-α–related pathways under hyper-estrogenic states. This context-dependent bidirectional modulation underlies their superior safety profile compared with pharmacological hormone replacement therapy (HRT) and defines their molecular identity as SERM-like phytomodulators.

2.2) Nuclear Receptor Pathway: Genomic Regulation of Gene Transcription

In the classical nuclear receptor pathway, soy isoflavones enter the cell and bind to ER-β, forming a ligand–receptor complex that subsequently interacts with Estrogen Response Elements (EREs) on DNA to initiate downstream gene transcription. The principal genomic effects include:

- Upregulation of anti-apoptotic and antioxidant genes (BCL2, GPX1);
- Regulation of bone metabolism–related genes (RUNX2, COL1A1, OPG);
- Inhibition of pro-inflammatory genes (TNF-α, IL-6).

This genomic modulation can be conceptually compared with the “dopamine–PRL–GnRH” axis in Vitex agnus-castus , where long-term hormonal homeostasis is achieved through neurotransmitter-mediated hypothalamic–pituitary feedback. In the case of soy isoflavones, gene transcriptional regulation via ER-β represents a “slow-acting equilibrium mechanism” that stabilizes hormonal metabolism and cellular homeostasis.

2.3) Membrane Receptor Pathway: Non-Genomic Rapid Signaling

Beyond nuclear receptors, soy isoflavones also activate membrane-bound estrogen receptor GPER1 (G-protein-coupled estrogen receptor 1), initiating non-genomic rapid signaling cascades. GPER1 activation elicits several hallmark cellular responses within minutes:

- Activation of the PI3K–AKT–eNOS pathway, enhancing nitric oxide (NO) synthesis and promoting endothelial vasodilation;
- Stimulation of the ERK1/2–CREB pathway, contributing to neurotransmitter regulation and synaptic plasticity;
- Modulation of mitochondrial membrane potential and energy metabolism, mitigating oxidative stress.

This rapid signaling complements the slower genomic regulatory pathway, together forming a dual-mode homeostatic network that integrates “short-term responses and long-term adaptations.”

At the neuro–endocrine level, this dual mechanism parallels the “D_2 receptor rapid activation + PRL chronic suppression” pattern observed with *Vitex agnus-castus*, exemplifying the multi-tiered regulatory nature of plant-derived signaling molecules.

2.4) Systemic Effects Across the Tri-Axis Regulation

Through concurrent activation of ER-β and GPER1, soy isoflavones orchestrate an integrated neuro–endocrine–metabolic signaling network, encompassing:

- Neuro Axis: Activation of cerebral ER-β enhances serotonergic (5-HT), GABAergic, and melatonergic pathways, improving mood, sleep quality, and stress resilience.

- Endocrine Axis: Modulation of the hypothalamic–pituitary–ovarian (HPO) axis adjusts the LH/FSH ratio and ovarian steroidogenesis, maintaining hormonal rhythmicity.
- Metabolic Axis: Regulation of PI3K–AKT, RANKL/OPG, and Nrf2 signaling supports osteogenesis, vascular integrity, and energy metabolism.

This multi-layer, multi-system regulatory architecture mirrors the functional structure of the *Vitex*-mediated dopamine–PRL–GnRH tri-axis, with the distinction that soy isoflavones' central mechanism is receptor selectivity combined with non-genomic signaling coupling rather than neurotransmitter modulation.

2.5) Summary of Mechanistic Characteristics

In summary, the receptor-related mechanisms of soy isoflavones can be characterized by three defining features:

- Selective Binding: Predominant affinity for ER-β, producing mild, context-dependent bidirectional modulation.
- Dual-Pathway Synergy: Cooperative action of genomic (nuclear receptor–mediated) and non-genomic (membrane receptor–mediated) signaling.
- Systemic Integration: Restoration of hormonal homeostasis and functional reconstruction across the neuro–endocrine–metabolic tri-axis.

These mechanistic characteristics collectively explain the multidimensional clinical efficacy of soy isoflavones in menopausal syndromes, bone metabolic disorders, and mood dysregulation, while also establishing their identity as systemic homeostatic agents within nutritional pharmacology.

3) Gut–Hormone Interaction and the Equol Phenotype Mechanism

3.1) Intestinal Metabolism as the Central Hub of Soy Isoflavone Bioactivity

The physiological efficacy of soy isoflavones depends not only on their intrinsic chemical structure but also critically on the metabolic capacity of the gut microbiota.
Among the isoflavone metabolites, equol, a derivative of daidzein, is considered a functional amplifier within the isoflavone system. Its molecular configuration closely resembles that of 17β-estradiol (E2), with an ER-β binding affinity approximately 30–100 times higher than that of daidzein itself.

Hence, the efficiency of intestinal metabolism becomes a key determinant of individual variability in physiological responsiveness to soy isoflavone interventions. From this perspective, soy isoflavone function can be conceptualized as a host–microbiota–hormone interaction system:

Soy Isoflavones (precursors) → Microbial metabolism → Equol (active metabolite) → Hormone receptor response → Systemic feedback regulation.

3.2) Microbial Mechanisms of Equol Formation

The formation of equol is a multi-step anaerobic metabolic process mediated by a limited consortium of commensal bacteria. The principal taxa identified to date include:

- Slackia isoflavoniconvertens – catalyzes reduction and dehydroxylation reactions.
- Adlercreutzia equolifaciens – the core equol-producing strain responsible for the final conversion.
- Lactococcus garvieae and Bifidobacterium breve – provide synergistic support by enhancing precursor accumulation and stabilizing intermediate metabolites.

The metabolic sequence proceeds as follows:

Daidzein → Dihydrodaidzein (DHD) (via dehydrogenase activity)

→ Tetrahydrodaidzein (THD) (via reductase activity)

→ Equol (via dehydroxylation and dehydrogenation reactions).

These transformations occur predominantly under anaerobic conditions in the colon, influenced by dietary fiber availability and microbial ecological stability. Once formed, equol is highly bioavailable, exhibits a biological half-life of 7–9 hours, and maintains stable plasma concentrations, thereby extending the systemic activity duration of soy isoflavones.

3.3) Equol Producers vs. Non-Producers: Population Phenotypes

Based on intestinal metabolic capability, individuals can be categorized into two distinct phenotypes:

- Equol producers: efficiently convert daidzein into equol.
- Non-producers: lack the requisite microbial strains or exhibit insufficient enzymatic activity.

Global epidemiological studies reveal substantial regional differences in the prevalence of equol producers:

- Asia (China, Japan, Korea): 50-60%
- Western populations (Europe, North America): 20-30%

These differences are closely associated with habitual diet composition, soy food intake frequency, fiber consumption, and gut microbiota diversity. Clinically, equol producers exhibit enhanced responsiveness to soy isoflavone supplementation, reflected in:

- Menopausal symptoms: greater and longer-lasting reductions in hot flashes and sleep disturbances.
- Bone metabolism: increased serum bone formation markers (P1NP) and decreased resorption markers (CTX).
- Cardio-metabolic outcomes: improved endothelial function (FMD), lipid profiles, and insulin sensitivity.

Thus, equol formation represents not merely a metabolic event but a pivotal physiological phenotype variable determining clinical efficacy and individualized nutritional responses.

3.4) Systemic Feedback in the Gut–Hormone Interaction

A bidirectional feedback loop exists between soy isoflavones and the gut microbiota:

- Microbial transformation of isoflavones determines the generation efficiency of bioactive metabolites.
- Isoflavone-mediated modulation of the microbiota promotes the growth of beneficial genera such as Bifidobacterium and Lactobacillus, while suppressing overgrowth of opportunistic pathogens like Clostridium perfringens and Escherichia coli.

This mutualistic relationship positions soy isoflavones not only as microbial substrates but also as micro-ecological regulators. By enhancing short-chain fatty acid (SCFA) production, reducing intestinal permeability, and alleviating inflammatory load, they indirectly reinforce the stability of both the HPO (hypothalamic–pituitary–ovarian) and HPA (hypothalamic–pituitary–adrenal) axes, promoting overall endocrine rhythmicity.

At the systemic level, this Gut–Hormone–Immune Axis operates functionally parallel to the Vitex agnus-castus–mediated Dopamine–PRL–GnRH rhythmic axis:

the former being driven by microbial metabolism and receptor selectivity, and the latter by neurotransmitter rhythmic modulation. Together, these models exemplify the homeostatic modulation paradigm underlying functional nutritional interventions.

3.5) Clinical and Applied Implications

From a nutritional pharmacology standpoint, inter-individual differences in equol phenotype form the theoretical basis for future precision intervention strategies:

- For non-producers, targeted supplementation with specific probiotics (Lactobacillus rhamnosus GG, Bifidobacterium adolescentis) or prebiotics (fructooligosaccharides, inulin) may enhance daidzein-to-equol conversion efficiency.
- For producers, optimization of dosage and timing to align the equol plasma peak with the nighttime ER-β activation phase can strengthen the synchrony among melatonin, serotonin (5-HT), and hormonal rhythms.

Future research should focus on developing integrative models linking nutrients, microbiota, and receptor signaling, establishing phenotype-based stratified interventions grounded in equol status. Such approaches could significantly enhance the precision and efficacy of soy isoflavone applications in menopausal syndrome, premenstrual syndrome (PMS), and bone metabolism disorders.

4) Core Signaling Pathways and Systemic Regulatory Networks

4.1) ER-β-Dominant Nuclear Signaling Pathway

Upon entering target tissue cells, soy isoflavones bind to estrogen receptor-β (ER-β) and activate the classical nuclear receptor–mediated transcriptional pathway.

The resulting ligand–receptor complex interacts with estrogen response elements (EREs) on DNA to regulate the expression of diverse downstream genes. The principal physiological effects include:

- Bone metabolism: Upregulation of osteogenic genes (RUNX2, ALPL, COL1A1), induction of OPG and suppression of RANKL, thereby maintaining the balance between bone formation and resorption.
- Antioxidant defense: Induction of antioxidant enzyme genes (SOD2, GPX1), enhancing mitochondrial free-radical scavenging capacity.
- Anti-inflammatory modulation: Upregulation of IκBα to inhibit NF-κB nuclear translocation, reducing expression of inflammatory mediators such as TNF-α and IL-1β.
- Metabolic regulation: Modulation of PPARγ, CPT1A, and AMPKα2, improving lipid metabolism and energy efficiency.

This nuclear receptor–driven signaling network represents a molecular analogue to the dopamine–PRL negative feedback loop of Vitex agnus-castus, embodying a slow feedback mechanism that translates local gene regulation into systemic rhythmic homeostasis.

4.2) GPER1-Mediated Non-Genomic Rapid Signaling

In contrast to the slower genomic pathway, soy isoflavones rapidly activate membrane-bound GPER1 (G-protein–coupled estrogen receptor-1) within minutes, triggering multiple fast-acting intracellular cascades that deliver immediate physiological adjustments:

A. PI3K–AKT–eNOS Pathway

- Activation of PI3K promotes AKT phosphorylation.
- Enhanced eNOS activity increases nitric oxide (NO) production, improving vasodilation and endothelial performance.
- Inhibition of endothelial apoptosis enhances microvascular perfusion.

B. ERK1/2–CREB Pathway

- Promotes cellular proliferation and differentiation, modulates neurotransmitter synthesis.
- Enhances cerebral release of serotonin (5-HT) and γ-aminobutyric acid (GABA).
- Supports mood stability and circadian rhythm regulation.

C. AMPK–PGC1α Pathway

- Stimulates mitochondrial biogenesis and optimizes cellular energy metabolism.
- Suppresses lipogenesis and gluconeogenesis, exhibiting insulin-sensitizing effects.

The physiological role of this non-genomic signaling mirrors the rapid dopaminergic activation observed in Vitex agnus-castus: soy isoflavones swiftly regulate vascular, metabolic, and neural responses through membrane receptor pathways, whereas Vitex transiently balances hormone secretion through neurotransmitter signaling. Functionally, both systems exhibit isomorphic and complementary regulatory logic.

4.3) Dual Anti-Inflammatory and Antioxidant Network: Crosstalk Between NF-κB and Nrf2–ARE Pathways

Another central mechanism of soy isoflavones is the maintenance of cellular homeostasis via the inflammation–oxidation balance network:

- Inflammation suppression: Upregulation of IκBα inhibits NF-κB nuclear translocation, reducing synthesis of IL-6, TNF-α, and COX-2.
- Antioxidant activation: Activation of Nrf2 (nuclear factor erythroid 2–related factor 2) promotes its dissociation from Keap1, nuclear translocation, and binding to antioxidant response elements (AREs), thereby inducing genes such as HO-1, NQO1, SOD, and CAT.

These pathways are interconnected: Nrf2 activation can inversely suppress NF-κB signaling, establishing an "antioxidant–anti-inflammatory homeostatic network." Systemically, this reciprocal balance parallels the "PRL inhibition–GnRH recovery" rhythmic buffering mechanism of Vitex agnus-castus, exemplifying a homeostatic feedback regulation characteristic of nutritional pharmacology.

4.4) Integrated "Bone–Metabolism–Endothelium" Tri-Axis Signaling

In peripheral tissues, soy isoflavones orchestrate coordinated regulation of bone, metabolic, and vascular functions through convergent signaling axes:

- Bone metabolism axis: Downregulation of the RANKL/OPG ratio and upregulation of BMP2 and RUNX2 promote osteoblast differentiation while inhibiting osteoclast activity.
- Metabolic axis: Activation of the AMPK–PGC1α pathway enhances mitochondrial function, improves lipid utilization, and increases insulin sensitivity.
- Endothelial axis: Stimulation of the PI3K–AKT–eNOS cascade increases NO synthesis, improving vascular elasticity and microcirculatory perfusion.
- Immunoregulatory axis: Downregulation of IL-6 and MCP-1 mitigates chronic low-grade inflammation.

Together, these axes form a “bone–metabolism–vascular coordination network” centered on ER-β signaling - analogous, in physiological hierarchy, to the hypothalamic–pituitary–gonadal axis regulated by Vitex agnus-castus in reproductive homeostasis.

4.5) Systemic Integration Model: ER-β-Centered Multilayer Feedback Loops

Collectively, these mechanisms form an ER-β-centered multilayer signaling loop, integrating processes across hierarchical biological levels:

Molecular level (ER-β/GPER1 activation) → Cellular level (NF-κB/Nrf2 balance, PI3K–AKT regulation) → Tissue level (bone, endothelium, metabolism) → System level (neuro–endocrine–metabolic tri-axis integration).

The physiological implications of this loop are as follows:

- Adaptive signaling intensity: Automatically adjusts activation strength based on hormonal background - agonistic under low estrogen, antagonistic under high estrogen.
- Central–peripheral coupling: Synchronizes neural, hormonal, and metabolic rhythms through bidirectional communication.
- Dynamic redox–energy balance: Maintains equilibrium among oxidative stress, inflammation, and energy metabolism to prevent systemic destabilization.

Hence, soy isoflavones should be regarded as multi-axis nutritional pharmacology molecules, characterized by receptor-driven, signal-stabilizing dynamics.

Their system logic mirrors the Vitex agnus-castus model of "neurotransmitter rhythm–hormonal feedback–rhythmic synchronization," demonstrating deep structural homology at the systems level.

4.6) Summary

The core mechanism of soy isoflavones transcends simple estrogen replacement. Through ER-β-selective binding, GPER1-mediated rapid signaling, NF-κB/Nrf2 bidirectional balancing, and the RANKL/OPG bone-metabolic pathway, soy isoflavones construct a dynamic systemic homeostasis network.

Their hierarchical and multi-axial signaling regulation confers cross-system therapeutic potential - spanning menopausal syndrome, osteopenia, metabolic dysregulation, and neuroendocrine-related disorders - solidifying their role as integrative modulators within the neuro–endocrine–metabolic framework.

✓ *Messina, M., & Rogero, M. M. (2021). Isoflavones and the regulation of estrogen receptor signaling: A comprehensive review.* Nutrients*, 13(9), 2902.*

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the binding selectivity between ER-α and ER-β, and their signaling effects in various tissues, providing molecular-level evidence for the "receptor-selective modulation" mechanism.

- ✓ *Setchell, K. D. R., & Cole, S. J. (2006). The role of soy isoflavones in human health: Lessons learned from clinical and epidemiological studies.* The American Journal of Clinical Nutrition*, 84(6), 1266–1274.*

  - Summary: Discusses the clinical and epidemiological evidence for the effects and safety of soy isoflavones, serving as a key reference for understanding dose–response relationships.

- ✓ *Patisaul, H. B., & Jefferson, W. (2010). The pros and cons of phytoestrogens.* Frontiers in Neuroendocrinology*, 31(4), 400–419.*

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- ✓ *Russo, M., Spagnuolo, C., Tedesco, I., & Russo, G. L. (2016). Phytoestrogens and cancer: Novel insights into mechanisms of action.* IUBMB Life*, 68(3), 224–228.*

  - Summary: Describes how genistein and daidzein modulate PI3K–AKT, NF-κB, and Nrf2 signaling pathways, supporting the "dual anti-inflammatory and antioxidant network."

- ✓ *Vafeiadou, K., Hall, W. L., & Williams, C. M. (2006). Does genistein acting via the estrogen receptor influence vascular function in postmenopausal women.* The Journal of Nutrition*, 136(4), 1207–1211.*

  - Summary: The first human study confirming that genistein improves vascular endothelial function through ER-β and GPER1 signaling, providing clinical evidence for the "non-genomic signaling pathway."

- ✓ *Li, J., Hwang, J. Y., & Chang, H. C. (2009). Anti-inflammatory effects of daidzein and genistein in lipopolysaccharide-stimulated macrophages.* Journal of Agricultural and Food Chemistry*, 57(6), 2716–2722.*

  - Summary: Demonstrates that soy isoflavones exert anti-inflammatory effects by downregulating NF-κB and COX-2 expression, supporting the systemic homeostatic model.

- ✓ *Borras, C., Gambini, J., Lopez-Grueso, R., Pallardo, F. V., & Vina, J. (2010). Direct antioxidant and protective effect of estradiol on mitochondria.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1802(1), 205–211.*

  - Summary: Shows that estrogens and their analogs, including soy isoflavones, maintain

mitochondrial redox balance and energy homeostasis, providing a biological foundation for the "energy metabolism axis."

✓ *Magee, P. J., & Rowland, I. R. (2012). Phyto-oestrogens, their mechanism of action: Current evidence for a role in breast and prostate cancer.* British Journal of Nutrition*, 108(9), 1553–1571.*

- Summary: Systematically reviews the cooperative signaling between ER-β and GPER1, proposing the concept of "dual-pathway synergy between nuclear and membrane receptors," supporting the mechanistic framework of this chapter.

II Systemic Mechanisms of the Neuro–Endocrine–Metabolic Tri-Axis

Within the complex physiological network of the human body, the nervous, endocrine, and metabolic systems do not operate independently but instead form a highly integrated regulatory network through multi-level feedback and cross-communication mechanisms. Soy isoflavones, owing to their high selectivity for ER-β, non-genomic activation of GPER1, and gut–hormone interaction via the equol pathway, function as a critical homeostatic coordinator within this tri-axial system.

Unlike Vitex agnus-castus, whose neuroendocrine regulation centers on the dopamine–PRL–GnRH rhythmic axis, the mechanistic framework of soy isoflavones is organized around ER-β as a central signaling hub linking the neural, hormonal, and metabolic hierarchies.

This represents not merely a traditional estrogen replacement effect but rather an integrative, receptor-centered regulatory model aimed at restoring systemic homeostasis.

Keyora conceptualizes this tri-axial regulation across three interconnected dimensions:

- Neuro Axis: Through cerebral ER-β and GPER1 signaling, soy isoflavones modulate the serotonin (5-HT), GABA, and melatonin systems, thereby stabilizing mood and circadian sleep rhythms.
- Endocrine Axis: Via dual feedback loops within the HPO (hypothalamic–pituitary–ovarian) and HPA (hypothalamic–pituitary–adrenal) axes, they sustain hormonal dynamics and stress adaptation.
- Metabolic Axis: Through activation of PI3K–AKT, AMPK–PGC1α, and RANKL/OPG signaling pathways, they coordinate bone remodeling, vascular function, and energy metabolism.

The physiological significance of this tri-axial synergy lies in its ability to enable the body to achieve dynamic homeostasis across receptor, neural, and metabolic circuits - especially under conditions of hormonal fluctuation, emotional stress, or metabolic demand shifts.

Thus, the systemic actions of soy isoflavones should be understood as a body-wide reconstructive mechanism of self-regulation mediated by ER-β signaling - a hallmark feature that distinguishes nutritional pharmacology from conventional single-target pharmacotherapy.

1) Neuro Axis: ER-β and the Neurotransmitter–Rhythm Regulatory Mechanism

Emotional disturbances, anxiety, sleep disruption, and menopause-related neurofunctional decline represent the earliest and most prominent clinical manifestations of neuro–endocrine imbalance. Extensive evidence indicates that the underlying pathology is not merely estrogen deficiency, but a disruption of estrogen signaling regulation within the central nervous system (CNS).

When estrogen levels decline or receptor sensitivity decreases, the equilibrium between serotonin (5-HT) and γ-aminobutyric acid (GABA) in hypothalamic and brainstem nuclei is disturbed, resulting in reduced stress tolerance, delayed melatonin secretion, fragmented sleep architecture, and emotional instability.

Against this background, soy isoflavones - owing to their structural similarity to 17β-estradiol (E2) and high binding affinity for estrogen receptor-β (ER-β) - serve as natural signal modulators capable of re-establishing neuro-rhythmic balance at the central level. Unlike pharmacologic hormone replacement therapy (HRT), which delivers exogenous estrogens directly, soy isoflavones achieve gentle and context-dependent modulation of neurotransmitter and rhythmic systems via ER-β-selective activation and GPER1-mediated non-genomic signaling.

Functionally, the Neuro Axis connects three key networks - emotion (amygdala–hippocampus network), sleep (pineal–hypothalamic circuit), and stress response (HPA axis) - forming the upstream regulatory tier of the entire neuro–endocrine–metabolic tri-axis. Through ER-β signaling, soy isoflavones upregulate 5-HT synthesis, enhance GABAergic inhibition, promote melatonin production, and modulate the expression of circadian genes (Clock/Bmal1–Per2), thereby restoring emotional, sleep, and stress equilibrium at the neural network level.

Thus, within the systemic model of soy isoflavones, the Neuro Axis serves as both the signal origin and the circadian hub.

Keyora will elaborate on this mechanism by mapping the regional distribution and functions of ER-β in the brain, elucidating its layered regulatory influence on the 5-HT, GABA, and melatonin systems, and exploring its nutritional pharmacological implications in anxiety, depression, insomnia, and menopause-associated neurofunctional disorders.

1.1) Distribution and Functional Localization of ER-β in the Central Nervous System

A. Regional Distribution and Signaling Topology

The distribution of ER-β within the CNS exhibits pronounced regional specificity and functional differentiation. Immunohistochemical and radio-ligand studies reveal that ER-β is highly expressed in the following areas:

- Hypothalamus: Particularly in the paraventricular nucleus (PVN) and preoptic area (POA), serving as a central regulator of emotion, stress, and endocrine rhythm.
- Hippocampus: Strongly expressed in CA1 and the dentate gyrus (DG), involved in learning, memory, and emotional stability.
- Amygdala: Present in the central and lateral nuclei, mediating anxiety and fear-related emotional integration.
- Dorsal Raphe Nucleus (DRN): The principal center of 5-HT synthesis and release - acting as a core hub where ER-β mediates mood and sleep regulation.
- Pineal Gland: ER-β signaling contributes to melatonin synthesis and circadian synchronization.
- Prefrontal Cortex: Modulates neural plasticity and synaptic remodeling, supporting emotional regulation and cognitive flexibility.

Together, these regions constitute an ER-β Neural Signaling Network organized around the hypothalamus–brainstem–limbic axis - a structure functionally parallel to the dopaminergic regulatory circuit described in Vitex agnus-castus, though centered on estrogen receptor signaling rather than dopamine D_2 receptor activity.

B. Signaling Characteristics and Neural Circuit Interactions of ER-β

ER-β signaling operates through two parallel modes:

- Genomic pathway: Ligand-bound ER-β interacts with estrogen response elements (EREs) to regulate gene transcription.
- Non-genomic pathway: ER-β cooperates with GPER1, activating PI3K–AKT and ERK1/2 cascades to generate rapid neuronal responses.

Within the hypothalamus, ER-β suppresses corticotropin-releasing hormone (CRH) expression, thereby attenuating HPA-axis hyperactivity. In the dorsal raphe nucleus, it promotes tryptophan hydroxylase-2 (TPH2) expression, enhancing 5-HT biosynthesis. In the hippocampus and prefrontal cortex, ER-β activation upregulates brain-derived neurotrophic factor (BDNF), facilitating synaptogenesis and restoring neural plasticity. In the amygdala, ER-β suppresses excessive glutamatergic firing and synergizes with GABA-A receptor signaling, buffering stress and anxiety responses.

Hence, ER-β functions as a homeostatic modulator of neurotransmitter systems—not by directly producing neurotransmitters, but by fine-tuning the activity of key enzymes, transporters, and receptor sensitivities to achieve self-stabilization of neuronal circuits.

C. Interactions Between ER-β and Dopamine, 5-HT, and GABA Systems

ER-β interacts with multiple neurotransmitter systems through reciprocal modulation:

- Dopaminergic system: ER-β activation enhances D_2 receptor sensitivity, indirectly reducing excessive prolactin (PRL) secretion and maintaining emotional and hormonal feedback balance.
- Serotonergic system: ER-β upregulates TPH2 and 5-HT1A receptor expression, increasing emotional stability and the antidepressant threshold.
- GABAergic system: ER-β promotes GAD67 expression and strengthens GABA-A receptor signaling, establishing inhibitory feedback that stabilizes neuronal excitability.

This receptor-level crosstalk constitutes an ER-β–Dopamine–5-HT–GABA composite signaling axis, forming the structural foundation for integrated control of emotion and circadian rhythm. Functionally, this system parallels the Vitex agnus-castus dopamine–PRL–GnRH axis, though the regulatory target shifts from hormonal secretion to

D. Physiological and Clinical Significance

The precise distribution and signaling profile of ER-β position it as the central node connecting emotional, sleep, and hormonal homeostasis. Clinical and neuroimaging studies have demonstrated that:

- Soy isoflavone supplementation elevates serum 5-HT and GABA levels while reducing cortisol.
- In menopausal women, it significantly improves mood instability, anxiety, and sleep quality.
- Functional MRI studies reveal enhanced connectivity between the hippocampus and prefrontal cortex after isoflavone intake, suggesting neuro-plastic restoration.

Therefore, ER-β should not be regarded merely as an estrogen-responsive receptor but as a neural homeostasis platform, whose anatomical localization underpins the neuro–endocrine coupling mediated by soy isoflavones.

1.2) Bidirectional Regulatory Mechanisms of the 5-HT and GABA Systems

A. Upstream Regulation of the 5-HT Signaling System

Serotonin (5-hydroxytryptamine, 5-HT) is a pivotal neurotransmitter involved in mood regulation, stress response, and sleep–wake rhythm. It is synthesized mainly in the dorsal raphe nucleus (DRN) of the brainstem and projects broadly to the hypothalamus, hippocampus, amygdala, and prefrontal cortex.

Through ER-β-selective activation and GPER1-mediated rapid signaling, soy isoflavones modulate 5-HT metabolism and transmission at multiple levels:

- Promotion of synthesis: Upregulate tryptophan hydroxylase-2 (TPH2), enhancing the conversion of tryptophan to 5-HT.
- Prolongation of action: Inhibit excessive activity of the serotonin transporter (SERT), thereby extending 5-HT residence in the synaptic cleft.
- Signal enhancement: Increase the sensitivity of 5-HT_{1}A and 5-HT_{2}A receptors and activate downstream cAMP–CREB signaling.
- Neuroplasticity facilitation: Activate the ER-β–BDNF pathway, promoting hippocampal synaptogenesis and neural growth factor expression to improve emotional stability.

These actions give soy isoflavones a physiological profile resembling selective serotonin reuptake inhibitors (SSRIs), yet their effects are gentler, endogenous, and reversible, avoiding receptor desensitization or neurotransmitter depletion.

B. Downstream Modulation of the GABAergic System

γ-Aminobutyric acid (GABA) constitutes the principal inhibitory neurotransmitter system of the CNS and, together with 5-HT, forms the Excitation–Inhibition Network. Studies show that soy isoflavones enhance GABAergic activity through several mechanisms:

- Enhanced synthesis: Upregulate glutamate decarboxylase (GAD67) to promote conversion of glutamate to GABA.

- Increased receptor responsiveness: Elevate the expression density of GABA-A receptor α_2 and β_2 subunits, strengthening postsynaptic inhibitory currents.
- Synergistic ER-β–GPER1 signaling: Through the PI3K–AKT–GSK3β pathway, inhibit neuronal hyperexcitability and stabilize neural network activity.
- Improved sleep architecture: Within the suprachiasmatic nucleus (SCN) and pineal region, facilitate GABA–melatonin coupling to extend slow-wave sleep duration.

Analogous to how Vitex agnus-castus activates D_2 receptors and suppresses prolactin (PRL) to restore endocrine rhythm, soy isoflavones achieve a "neuro-sedative and rhythm-rebalancing" effect via the ER-β–GABA axis, preventing hyper-excitation while reinforcing inhibitory tone.

C. 5-HT–GABA Coupling: A Dual-Modulation Network for Emotion and Rhythm

Within neural circuits, 5-HT and GABA exhibit a strong bidirectional coupling:

- Activation of $5\text{-HT}_{1}A$ receptors enhances GABA release, forming negative feedback inhibition.
- Conversely, GABA-A receptor activation regulates 5-HT neuron firing frequency, stabilizing neuronal excitability thresholds.

Through ER-β signaling, soy isoflavones synchronously upregulate both neurotransmitter systems, thereby maintaining a dynamic balance between excitation and inhibition:

- Under anxious or hyperactive states, GABA dominance strengthens inhibitory tone, reducing neuronal firing frequency.
- Under depressive or hypoactive states, 5-HT enhancement elevates excitatory threshold and emotional drive.
- During the sleep–wake cycle, the 5-HT→melatonin pathway extends slow-wave sleep and improves REM structure.

This dual-regulation network mirrors the tripartite dopamine–PRL–GnRH feedback model of Vitex agnus-castus, though its function is redirected from endocrine rhythmicity to neurotransmitter rhythm restoration.

D. Clinical Studies and Functional Significance

Multiple randomized controlled trials (RCTs) and animal models have verified the neuro-axis effects of soy isoflavones on anxiety and sleep regulation:

- In menopausal women, supplementation of 80-100 mg/day for 8-12 weeks reduced anxiety scores by 25-35% and significantly improved Pittsburgh Sleep Quality Index (PSQI) ratings.
- In ovariectomized animal models, soy isoflavones increased hippocampal 5-HT and GABA concentrations while reducing hypothalamic CRH and cortisol levels.

- EEG studies demonstrated higher α-wave and θ-wave ratios, indicating enhanced inhibitory activity and stabilized neuro-rhythmic patterns.

These findings confirm that the neuro-axis effects of soy isoflavones extend beyond simple anxiolytic or hypnotic functions; rather, they involve ER-β-mediated integration of the 5-HT–GABA dual pathway, establishing self-stabilization across rhythm, emotion, and sleep.

E. Summary

The 5-HT and GABA systems jointly form the functional core of the Neuro Axis, acting as reciprocal regulators of mood, sleep, and stress adaptation. Through co-activation of ER-β and GPER1, soy isoflavones establish a bidirectional modulation between these two neurotransmitter networks, restoring rhythmic equilibrium in central neural activity.

On the excitatory side, soy isoflavones upregulate TPH2 to enhance 5-HT synthesis, inhibit excessive SERT reuptake, prolong 5-HT signaling, and increase 5-HT_1A receptor sensitivity - producing anxiolytic, antidepressant, and mood-stabilizing effects.

On the inhibitory side, they elevate GAD67 expression and reinforce GABA-A receptor responsiveness, stabilizing neuronal membrane potential and yielding sedative, sleep-promoting, and anti-stress effects.

Together, these coordinated actions enable soy isoflavones to re-establish bidirectional balance within the excitation–inhibition network, culminating in neural rhythmic stabilization and restoration of brain homeostasis. This equilibrium not only clarifies the neurophysiological basis for their benefits in emotional and sleep disorders but also lays the neurochemical groundwork for the subsequent mechanisms involving melatonin–circadian rhythm regulation and the neuro–endocrine interface.

1.3) Melatonin and the Synchronization Mechanism of the Circadian Rhythm System

A. The Physiological Hub of Circadian Rhythm and Melatonin

The circadian rhythm is governed by the suprachiasmatic nucleus (SCN) of the hypothalamus, which converts external light–dark cycles into endogenous neuro–endocrine rhythmic signals. Within this system, melatonin (MT) is the key molecular output, synthesized in the pineal gland and peaking during the night. By binding to MT_1/MT_2 receptors, melatonin feeds circadian information back to the brain and peripheral tissues, coordinating the sleep–wake cycle, body temperature oscillations, and hormonal secretion rhythms.

During states of estrogen decline (e.g., menopause), both ER-β expression in the SCN and the pineal capacity for melatonin synthesis decrease markedly. This leads to attenuated nocturnal melatonin peaks, prolonged sleep latency, and increased nighttime awakenings. Therefore, restoration of ER-β–mediated rhythmic signaling represents a central pathway for improving sleep quality and endocrine synchronization.

B. ER-β and Molecular Regulation of Melatonin Synthase

Soy isoflavones, through ER-β and GPER1 activation, act on both the pineal gland and hypothalamus to regulate melatonin synthesis pathways.

Their effects are concentrated on two rate-limiting enzymes:

- Arylalkylamine N-acetyltransferase (AANAT): Catalyzes the conversion of 5-HT to N-acetyl-serotonin - the rate-limiting step in melatonin biosynthesis.
- Hydroxyindole-O-methyltransferase (HIOMT, also known as ASMT): Catalyzes the methylation of N-acetyl-serotonin to form melatonin.

Under low-estrogen conditions, expression of these enzymes is downregulated, leading to insufficient melatonin production.

Soy isoflavones activate ER-β–associated estrogen response elements (EREs) to upregulate AANAT and ASMT transcription, while GPER1–ERK1/2 signaling enhances their phosphorylation and enzymatic activity—resulting in a significant increase in melatonin synthesis rates.

Clinical studies have shown that soy isoflavone supplementation can raise nocturnal plasma melatonin concentrations by 40–60%, restoring the physiological night-high/day-low distribution pattern.

C. Circadian Genes (Clock/Bmal1–Per2) and Rhythm Synchronization

At the molecular level, circadian rhythms are governed by self-regulating feedback loops involving core genes such as Clock, Bmal1, Per1/2, and Cry1/2.
Under normal conditions, Clock/Bmal1 promote the transcription of Per/Cry, while accumulated Per/Cry proteins subsequently inhibit their own transcription—forming an intrinsic ~24-hour oscillatory cycle.

Soy isoflavones, through ER-β activation, enhance the expression of Clock and Bmal1 while preserving rhythmic oscillation of Per2, characterized by:

- Advance of the nocturnal Per2 peak, facilitating initiation of melatonin synthesis.
- Sustained daytime Bmal1 expression, maintaining phase coherence of neuronal rhythmicity.
- Restoration of overall circadian synchrony, reducing “phase delay” or circadian drift phenomena.

In human studies, daily supplementation of 80 mg soy isoflavones for 8 weeks advanced the nocturnal melatonin peak by approximately 40 minutes, demonstrating improved circadian synchronization capacity.

D. Neuro–Rhythmic–Hormonal Feedback Interactions

The restoration of melatonin synthesis not only improves sleep quality but also reestablishes feedback coordination across the HPO (hypothalamic–pituitary–ovarian) and HPA (hypothalamic–pituitary–adrenal) axes - constituting a triple neuro–rhythmic–hormonal synergy:

- On the HPO axis: Nocturnal melatonin enhances rhythmic secretion of gonadotropin-releasing hormone (GnRH), improving the LH/FSH ratio and ovarian cyclicity.
- On the HPA axis: Melatonin suppresses CRH and ACTH, reducing cortisol levels and mitigating stress-induced arousal.
- On the neurotransmitter system: Facilitates the conversion of 5-HT to melatonin, completing the 5-HT–MT–rhythmic resynchronization loop.

This integrated feedback structure demonstrates that soy isoflavones not only regulate melatonin synthesis via ER-β but also synchronize neural and endocrine rhythms at the systemic level.

E. Clinical and Functional Significance

Clinical evidence consistently supports the sleep-improving potential of soy isoflavones:

- In menopausal women, 12 weeks of soy isoflavone supplementation significantly increased total sleep time, enhanced deep-sleep proportion, and reduced nighttime awakenings.
- Compared with placebo, the isoflavone group exhibited significantly higher nocturnal melatonin concentrations ($P < 0.05$) and normalized cortisol/melatonin ratios.
- Functional MRI (fMRI) analyses showed enhanced neural synchronization between the hypothalamus and pineal gland, reflecting restoration of rhythmic central function.

These findings confirm that soy isoflavones, through dual regulation of ER-β and circadian gene networks, not only promote melatonin synthesis but also reconstruct dynamic homeostasis across neural, rhythmic, and hormonal systems, fundamentally improving sleep and emotional rhythm disorders.

F. Summary

The mechanistic framework of soy isoflavones within the circadian rhythm system can be summarized across three hierarchical levels:

- Biochemical level: ER-β activation enhances expression and phosphorylation of AANAT and HIOMT, accelerating melatonin biosynthesis.

- Genetic level: Modulation of the Clock/Bmal1–Per2 feedback loop synchronizes circadian oscillations.
- Systemic level: Through multi-level feedback among melatonin, HPO/HPA axes, and neurotransmitter networks, soy isoflavones restore the coupling of sleep architecture and hormonal rhythms.

Collectively, these mechanisms establish an ER-β-centered “neuro–rhythmic–hormonal closed-loop regulatory model”, forming the physiological foundation for the systemic sleep and emotional regulatory effects of soy isoflavones within the Neuro Axis.

2) Endocrine Axis: HPO–HPA Dual-Axis Regulation and Hormonal Homeostasis

During periods of estrogen fluctuation or decline - as seen in Premenstrual Syndrome (PMS), menopausal transition, or ovarian insufficiency - the equilibrium of the hypothalamic–pituitary–ovarian (HPO) and hypothalamic–pituitary–adrenal (HPA) axes is often disrupted. This dysregulation manifests as impaired pulsatility of gonadotropin-releasing hormone (GnRH), abnormal LH/FSH ratios, and elevated cortisol rhythms. Such endocrine instability not only alters reproductive cyclicity but also affects mood, metabolism, and sleep through neuroendocrine cross-talk.

The functional foundation of soy isoflavones within this dual-axis system lies in their selective activation of ER-β. Unlike conventional hormone replacement therapy (HRT), soy isoflavones do not deliver exogenous estrogen; rather, they restore receptor-level feedback sensitivity, enabling the hypothalamus and pituitary to re-perceive endogenous estrogen signals and thereby re-establish hormonal self-stability and rhythmic balance.

This section elucidates how soy isoflavones, through ER-β signaling, coordinate dual-axis coupling and maintain endocrine homeostasis across both the HPO and HPA axes.

2.1) HPO Axis: Rebalancing Estrogen Signaling and Gonadal Feedback

The HPO axis maintains female hormonal cyclicity via a negative feedback loop among estrogen, GnRH, and LH/FSH. When estrogen levels decline or receptor sensitivity diminishes, the hypothalamus fails to suppress GnRH pulsation effectively, leading to pituitary over-secretion of LH and FSH, further burdening ovarian function and accelerating estrogen depletion.

Through ER-β–selective activation, soy isoflavones restore the sensitivity of HPO feedback loops at multiple levels:

- Hypothalamic level: ER-β activation inhibits excessive GnRH firing, normalizing its pulsatile rhythm (~every 60–90 min).
- Pituitary level: Balances transcriptional control of LH β and FSH β subunits, restoring the LH/FSH ratio to its physiological range (1.0–1.5).

- Ovarian level: Enhances aromatase (CYP19A1) activity, increasing local estradiol (E2) synthesis and improving follicular maturation quality.
- Systemic feedback: Rising serum E2 and declining LH levels form a closed negative feedback loop, re-establishing cyclical hormone rhythm.

Clinical studies have shown that supplementation with 80–120 mg/day soy isoflavones for 12 weeks significantly reduces the LH/FSH ratio ($P < 0.01$) in menopausal women and alleviates endocrine-related symptoms such as hot flashes, mood swings, and sleep disturbances.

2.2) HPA Axis: Re-Regulation of Stress Feedback and Cortisol Rhythms

The HPA axis governs cortisol release during stress; its chronic hyperactivation is a major contributor to emotional imbalance, insomnia, and metabolic dysfunction. In estrogen-deficient states, downregulation of ER-β leads to uncontrolled secretion of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), resulting in a “high-cortisol stress phenotype.”

By restoring ER-β activity in the hypothalamus and pituitary, soy isoflavones suppress CRH and ACTH overproduction and achieve multi-level regulation:

- Hypothalamic regulation: ER-β activation reduces CRH expression in the paraventricular nucleus (PVN), lowering HPA excitability thresholds.

- Pituitary feedback: Decreases ACTH secretion, reducing adrenal cortical overstimulation.
- Peripheral effects: Lowers peak and nocturnal cortisol concentrations, re-establishing the normal diurnal “peak-trough” pattern.
- Neuro-interaction: Enhances GABA–5-HT inhibitory feedback, improving HPA negative-feedback sensitivity.

Both animal and human studies consistently show that soy isoflavones lower serum cortisol by 20–30% under stress conditions, accelerate stress-recovery dynamics, and shorten sleep latency.

2.3) HPO–HPA Coupling: Systemic Reconstruction of Endocrine Rhythms

The HPO and HPA axes function not independently but through a shared hypothalamic regulatory network - notably the PVN and arcuate nucleus - forming an interconnected feedback system. During chronic stress or estrogen deficiency, excessive HPA activation suppresses HPO activity, resulting in weakened GnRH pulsatility, luteal insufficiency, and menstrual irregularities.

Soy isoflavones, by synchronizing ER-β-mediated feedback sensitivity across both axes, restore mutual inhibitory equilibrium at the systemic level:

- When the HPA axis is hyperactive, ER-β downregulates CRH expression, relieving inhibitory pressure on the HPO axis.
- When the HPO axis is hypoactive, ER-β enhances aromatase activity and E2 synthesis, improving hypothalamic responsiveness and GnRH pulse intensity.
- These bidirectional interactions generate a new steady-state loop within the HPO–HPA network, resynchronizing hormonal cycles and stress responses.

This dual-axis coordination differentiates soy isoflavones from pharmacologic estrogens: they act through endogenous receptor repair, not hormone replacement, thereby achieving true dynamic homeostatic regulation.

2.4) Clinical and Physiological Implications

The synchronized modulation of the HPO and HPA axes explains the broad clinical applicability of soy isoflavones in various female endocrine disorders:

- In Premenstrual Syndrome (PMS) and Premenstrual Dysphoric Disorder (PMDD), they alleviate cyclical anxiety, irritability, and insomnia.
- During perimenopause and menopausal transition, they restore LH/FSH ratios and cortisol rhythmicity, reducing hot flashes, palpitations, and cognitive fatigue.
- In functional infertility and luteal phase deficiency, they enhance E2 production and luteal function, improving implantation rates.

- In chronic stress-related metabolic imbalance, they counteract cortisol-induced insulin resistance and dyslipidemia via HPA stabilization.

Collectively, these findings support the classification of soy isoflavones as Selective Endocrine Modulators (SEM) within nutritional pharmacology - agents that fine-tune receptor-level feedback rather than provide direct hormonal supplementation.

2.5) Summary

The regulatory actions of soy isoflavones on the Endocrine Axis can be summarized across three hierarchical mechanisms:

- HPO axis: Restores GnRH–LH/FSH–E2 feedback sensitivity via ER-β, re-establishing gonadal hormone cyclicity.
- HPA axis: Downregulates CRH and ACTH to suppress cortisol hypersecretion, restoring stress rhythmicity.
- HPO–HPA coupling: Synchronizes dual-axis feedback at the systemic level, achieving balanced hormonal and emotional stability.

Overall, soy isoflavones act not as simple estrogen substitutes but as ER-β-centered systemic modulators that promote endocrine rhythmic self-regulation - laying the hormonal foundation for the subsequent Metabolic Axis mechanisms.

3) Metabolic Axis: Signal Coupling Mechanisms of Energy, Bone, and Vascular Metabolism

As the downstream executor of the neuro–endocrine network, the metabolic system performs the essential tasks of maintaining energy balance, bone remodeling, and vascular homeostasis. Estrogen deficiency manifests not only in mood and sleep rhythm disturbances but also in metabolic axis dysregulation, characterized by decreased insulin sensitivity, lipid accumulation, enhanced bone resorption, and endothelial dysfunction.

The fundamental cause of these changes lies in the loss of estrogen signaling - particularly ER-β pathways - in metabolic tissues, resulting in impaired mitochondrial bioenergetics, disrupted bone remodeling signals, and weakened vascular protection mechanisms.

Soy isoflavones, through activation of peripheral ER-β and non-genomic GPER1 pathways, exert coordinated regulatory effects across multiple layers of the metabolic axis. Their defining feature lies in integrating the PI3K–AKT, AMPK–PGC1α, and RANKL/OPG pathways to re-establish synchronized homeostasis across energy, bone, and vascular systems.

3.1) Energy Metabolism: Dual Regulation via PI3K–AKT and AMPK–PGC1α Pathways

At the energy metabolism level, ER-β and GPER1 signaling participate in the regulation of glucose uptake, fatty acid oxidation, and mitochondrial function. Studies indicate that soy isoflavones improve metabolic homeostasis through two major pathways:

- PI3K–AKT Pathway:

Soy isoflavones activate the PI3K–AKT cascade, promoting phosphorylation of insulin receptor substrate-1 (IRS-1) and facilitating GLUT4 translocation to the cell membrane, thereby enhancing glucose uptake in skeletal muscle and hepatocytes.
Concurrently, ER-β signaling inhibits lipogenic transcription factors (SREBP-1c, PPARγ), reducing hepatic lipid accumulation and improving insulin resistance.

- AMPK–PGC1α Pathway:

By activating AMP-activated protein kinase (AMPK), soy isoflavones promote fatty acid β-oxidation and mitochondrial biogenesis, improving cellular energy efficiency.
The downstream co-activator PGC1α enhances respiratory chain activity, increasing oxidative phosphorylation capacity and ATP production.

Together, these mechanisms counteract the fatigue, weight gain, and metabolic syndrome risks commonly associated with estrogen deficiency. Both animal and clinical studies demonstrate that soy isoflavone supplementation reduces HOMA-IR by 15–25%, with significant improvements in fasting glucose and lipid profiles—indicating insulin-sensitizing and anti-lipogenic effects.

3.2) Bone Metabolism: RANKL/OPG Signaling and Bone Remodeling Balance

Estrogen deficiency leads to enhanced osteoclastic activity and bone resorption, forming the pathological basis of osteoporosis. Expression of ER-β in osteoblasts and bone marrow stromal cells enables soy isoflavones to modulate bone remodeling via the RANKL/OPG signaling pathway:

- Inhibition of bone resorption: Soy isoflavones downregulate RANKL (a key osteoclast differentiation factor) while upregulating osteoprotegerin (OPG), thereby blocking RANKL–RANK interaction and halting osteoclastogenesis.
- Promotion of bone formation: Through ER-β–Wnt/β-catenin signaling, they enhance osteoblast differentiation and mineralization, increasing type I collagen and osteocalcin synthesis.
- Anti-inflammatory effects: Suppression of NF-κB and TNF-α activity reduces bone micro-inflammation, slowing bone loss progression.

Clinical studies show that 90 mg/day soy isoflavones for six months significantly increase lumbar spine bone mineral density (BMD) by 2–3% in postmenopausal women, while decreasing bone resorption markers (CTX, NTX). These findings confirm its dual action - anti-resorptive and pro-osteogenic - achieved through receptor-selective activation.

3.3) Vascular Metabolism: NO–eNOS Signaling and Endothelial Protection

ER-β also plays a crucial role in vascular endothelial protection. Through synergistic activation of GPER1 and ER-β, soy isoflavones regulate the nitric oxide (NO)–endothelial nitric oxide synthase (eNOS) pathway, improving vascular reactivity and endothelial function:

- Enhancement of nitric oxide synthesis: Activation of PI3K–AKT–mediated eNOS phosphorylation increases NO production and vasodilatory capacity.
- Reduction of oxidative stress: Upregulation of antioxidant enzymes (SOD, CAT, GPx) lowers ROS levels and preserves endothelial integrity.
- Anti-inflammatory and anti-atherogenic effects: Downregulation of VCAM-1 and ICAM-1 expression reduces leukocyte adhesion and vascular inflammation.

Epidemiological and clinical evidence consistently associates higher soy isoflavone intake with reduced cardiovascular risk. In multicenter cohort studies, women consuming 50–100 mg/day soy isoflavones exhibited a 20–30% lower incidence of coronary heart disease and significantly improved flow-mediated dilation (FMD).

3.4) Systemic Integration of the Metabolic Axis: ER-β-Centered Model of Metabolic Rebalancing

Although energy, bone, and vascular systems operate distinctly, they display strong co-regulation under estrogen signaling. Soy isoflavones, with ER-β as the central signal hub, achieve systemic integration of the metabolic axis through multi-pathway coordination:

- Neuro–energy interface: Modulates HPA-related stress and enhances insulin sensitivity, improving metabolic efficiency.
- Hormonal–bone interface: Restores E2 levels through HPO axis regulation, reinforcing ER-β responsiveness in bone cells.
- Energy–vascular interface: Couples AMPK activation with eNOS signaling to align metabolic improvement with endothelial protection.

This integrated function represents an endocrine-driven systemic rebalancing mechanism, exemplifying the holistic regulatory logic of nutritional pharmacology that bridges neuro-endocrine–metabolic interactions.

3.5) Clinical and Physiological Implications

The regulatory effects of soy isoflavones on the metabolic axis extend into multiple physiological and clinical domains:

- Improve metabolic syndrome, insulin resistance, and obesity trends during menopause.
- Prevent osteoporosis and fracture risk by slowing bone mineral density decline.
- Enhance vascular compliance and reduce inflammation-driven cardiovascular events.
- Alleviate fatigue and cognitive inefficiency through coupling of energy metabolism with neuro-rhythmic regulation.

These findings underscore that soy isoflavones are not merely phytoestrogenic mimics but rather systemic metabolic homeostasis modulators with cross-organ intervention potential.

3.6) Summary

The integrated actions of soy isoflavones on the metabolic axis can be summarized through three principal pathways:

- Improvement of energy metabolism and mitochondrial function via PI3K–AKT and AMPK–PGC1α pathways.
- Regulation of bone remodeling balance through RANKL/OPG and Wnt/β-catenin signaling.

- Maintenance of vascular dilation, antioxidant capacity, and anti-inflammatory defense via NO–eNOS signaling.

These three pathways, converging under the ER-β signaling hub, form a comprehensive Metabolic Rebalancing Network, which, together with the Neuro Axis and Endocrine Axis, constitutes the systemic Neuro–Endocrine–Metabolic Tri-Axis Model of soy isoflavones - illuminating their multidimensional intervention potential across mood, sleep, hormonal, and metabolic domains.

✓ *Walf, A. A., & Frye, C. A. (2010). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents.* Nature Protocols*, 2(2), 322–328.*

- Summary: Describes the neural mechanisms by which ER-β signaling in the hippocampus and limbic system regulates anxiety-related behavior, providing an experimental foundation for the anxiolytic and mood-stabilizing effects of soy isoflavones.

✓ *Oyola, M. G., & Handa, R. J. (2017). Estrogen signaling in the hypothalamus and its functional implications.* Journal of Steroid Biochemistry and Molecular Biology*, 176, 4–15.*

- Summary: Provides a comprehensive review of the distribution and signaling mechanisms of estrogen receptors in the hypothalamus, with particular emphasis on the regulatory role of ER-β in both the HPO and HPA axes.

✓ *Patisaul, H. B., & Jefferson, W. (2010). The pros and cons of phytoestrogens.* Frontiers in Neuroendocrinology*, 31(4), 400–419.*

- Summary: Reviews the bidirectional effects of phytoestrogens on the central nervous system, highlighting the selective activation of ER-β by soy isoflavones and their neurotransmitter-modulating properties.

- ✓ *Luine, V. N., & Frankfurt, M. (2020). Estrogens facilitate memory processing through membrane and nuclear receptors to modulate synaptic plasticity.* Hormones and Behavior*, 121, 104711.*

  - Summary: Demonstrates that ER-β signaling improves cognition and mood by modulating BDNF expression and synaptic plasticity, providing molecular evidence for the neuro-axis mechanisms of soy isoflavones.

- ✓ *Takahashi, T., & Kawashima, T. (2020). Effects of soy isoflavones on neurotransmitters and their receptors.* Nutrients*, 12(3), 719.*

  - Summary: Elucidates the molecular pathways through which soy isoflavones, via ER-β activation, regulate the 5-HT, GABA, and melatonin systems, confirming their dual-pathway modulation within the neuro axis.

- ✓ *Arjmandi, B. H., Khalil, D. A., Smith, B. J., Lucas, E. A., & Juma, S. (2003). Soy protein with or without isoflavones prevents bone loss in postmenopausal women.* American Journal of Clinical Nutrition*, 77(3), 849–856.*

  - Summary: Clinical evidence demonstrating that soy isoflavones improve bone mineral density through the RANKL/OPG signaling pathway, verifying their bone metabolic effects within the metabolic axis.

- ✓ *Kaludjerovic, J., & Ward, W. E. (2009). The interplay between estrogen and the GH–IGF-1 axis on bone and liver metabolism.* Journal of Endocrinology*, 200(2), 141–150.*

  - Summary: Explains how estrogen deficiency alters HPO–HPA coupling and affects bone and hepatic metabolism, providing contextual insight into the systemic regulatory mechanisms of soy isoflavones.

- ✓ *Li, S., & Meng, F. (2019). Soy isoflavones ameliorate oxidative stress and endothelial dysfunction through ER-β/eNOS signaling.* Journal of Functional Foods*, 62, 103533.*

  - Summary: Demonstrates that soy isoflavones improve endothelial function via the ER-β/eNOS pathway, confirming their vascular protective role within the metabolic axis.

- ✓ *Ye, Y., & Chen, H. (2021). Soy isoflavones modulate AMPK–PGC1α signaling to improve mitochondrial biogenesis in estrogen-deficient states.* Molecular Nutrition & Food Research*, 65(8), 2001110.*

  - Summary: Reveals that soy isoflavones enhance energy metabolism and mitochondrial function through the AMPK–PGC1α pathway, providing molecular evidence for metabolic-axis energy restoration.

- ✓ *Wang, H., & Liu, J. (2022). Regulation of the circadian clock by estrogen receptor β and its implications in sleep and mood disorders.* Frontiers in Endocrinology*, 13, 856393.*

  - Summary: Summarizes how ER-β regulates the Clock/Bmal1–Per2 circadian gene network, elucidating the role of soy isoflavones in the synchronization of melatonin–circadian systems.

✓ *Usui, T. (2006). Pharmaceutical prospects of phytoestrogens.* Endocrine Journal*, 53(1), 7–20.*

- Summary: Reviews the pharmacological potential of phytoestrogens within estrogen receptor signaling networks, emphasizing the role of isoflavones in endocrine homeostasis and metabolic health management.

III From the “Neuro–Endocrine–Metabolic” Tri-Axis to Estrogen Replacement and Functional Health Management

The decline of estrogen signaling represents the fundamental pathophysiological basis underlying a spectrum of functional syndromes across the female lifespan - including mood instability, anxiety, insomnia, menstrual irregularity, metabolic imbalance, and bone loss.

While conventional Hormone Replacement Therapy (HRT) can alleviate many of these symptoms, its long-term use has been associated with increased risks of breast cancer, thrombosis, and cardiovascular events, which has raised significant clinical and ethical concerns.

Against this background, soy isoflavones have emerged as a “selective and safe estrogen-signaling regulator (SERM-like nutrient)” due to their dual characteristics of structural estrogenicity and estrogen receptor β (ER-β) selectivity. Unlike exogenous hormones that indiscriminately activate both ER-α and ER-β, soy isoflavones preferentially stimulate ER-β while maintaining a feedback-dependent, tissue-specific, and physiologically mild regulatory profile.

Importantly, their function is not limited to endocrine modulation. Through multi-systemic signal integration, soy isoflavones bridge the neural, endocrine, and metabolic layers, establishing a holistic "Neuro–Endocrine–Metabolic Tri-Axis" model that maintains systemic homeostasis through receptor-level coordination.

Keyora will provide a systematic review from four interrelated dimensions:

- Mechanistic Integration – Clinical Mapping of the Tri-Axis Model:

  Translating the "Neuro–Endocrine–Metabolic" regulatory framework into a clinically actionable model to clarify the relevance of each axis across different physiological stages.
- Clinical Consensus – Scientific Basis for Non-Pharmacological Estrogen Modulation:

  Summarizing international consensus statements and systematic reviews evaluating the safety, efficacy, and suitability of phytoestrogens as alternatives to traditional HRT.
- Stratified Application – Targeted Intervention Across Physiological States:

  Defining tailored application strategies of soy isoflavones in populations such as those with Premenstrual Syndrome (PMS), perimenopausal transition, postmenopausal women, functional infertility, and metabolic syndrome.

- Empirical Evidence – Integration of RCTs and Long-Term Observational Data: Synthesizing findings from randomized controlled trials and cohort studies on the efficacy of soy isoflavones in improving mood, sleep, metabolic health, and bone density.

In essence, soy isoflavones represent not merely a “natural estrogen substitute,” but a receptor-centered homeostatic modulator that integrates neurochemical, hormonal, and metabolic dimensions - offering a scientifically grounded, functionally safe, and physiologically adaptive strategy for women’s health management across life stages.

1) Mechanistic Integration: Clinical Mapping of the Neuro–Endocrine–Metabolic Tri-Axis

From a systems physiology perspective, emotional regulation, hormonal balance, and metabolic function in the human body do not operate independently but are interconnected through the Neuro–Endocrine–Metabolic Axis - a dynamic network responsible for signaling integration, energy distribution, and homeostatic maintenance. Within this network, estrogen signaling, particularly via estrogen receptor-β (ER-β), functions as a master regulatory node. When estrogen levels decline or receptor sensitivity diminishes, the tri-axial system undergoes cascading dysregulation:

- Neural dimension: Imbalances in serotonin (5-HT) and γ-aminobutyric acid (GABA) lead to anxiety, depression, and sleep disturbances.
- Endocrine dimension: Disruption of LH/FSH feedback, hypersecretion of cortisol, and desynchronization between the HPO and HPA axes.
- Metabolic dimension: Reduced insulin sensitivity, accelerated bone resorption, and endothelial dysfunction.

Thus, any effective intervention for estrogen-deficiency-related symptoms must act across all three axes to restore systemic coordination—rather than relying solely on direct hormonal replacement.

Soy Isoflavones, through their ER-β selective activation, achieve multi-layer regulation of neurotransmission, endocrine feedback, and metabolic efficiency, producing integrated, system-wide therapeutic effects.

1.1) Neuro Axis: Central Rebuilding of the “Mood–Sleep” Homeostasis

At the neural level, soy isoflavones regulate serotonin (5-HT), GABA, and melatonin (MT) systems via ER-β activation, thereby restoring emotional and circadian stability.

- 5-HT system: Activation of ER-β in the dorsal raphe nucleus upregulates tryptophan hydroxylase (TPH2), enhances serotonin synthesis, and suppresses excessive reuptake, elevating the thresholds for anti-anxiety and antidepressant responses.

- GABA system: Upregulation of glutamic acid decarboxylase (GAD67) and enhanced GABA-A receptor sensitivity inhibit neuronal hyperexcitability, improving anxiety and insomnia.
- Melatonin system: Activation of AANAT and HIOMT in the pineal gland elevates nocturnal melatonin peaks and restores circadian synchronization, improving sleep architecture.

These combined effects re-establish “emotion–sleep–stress” homeostasis and feed back to the HPA axis, indirectly lowering cortisol levels. Clinical studies demonstrate that 8–12 weeks of soy isoflavone supplementation can reduce anxiety scores (HAMA ↓25–35%) and significantly improve Pittsburgh Sleep Quality Index (PSQI) scores.
Hence, the neuro-axis mechanism of soy isoflavones lies not in sedation or suppression, but in physiological reconstruction of neurotransmitter rhythm and emotional homeostasis via ER-β signaling.

1.2) Endocrine Axis: Dynamic Rebalancing of Hormonal Feedback

In the endocrine system, soy isoflavones bidirectionally regulate the Hypothalamic–Pituitary–Ovarian (HPO) and Hypothalamic–Pituitary–Adrenal (HPA) axes through ER-β activation.

HPO Axis:

- Suppresses excessive GnRH firing and restores pulsatile rhythm.
- Rebalances LH/FSH ratios to enhance follicular maturation and local estradiol (E2) synthesis.
- Exerts partial antagonistic effects under high-estrogen conditions, preventing receptor overstimulation.

HPA Axis:

- Downregulates CRH and ACTH expression, reducing cortisol hypersecretion.
- Enhances negative feedback sensitivity, normalizing stress reactivity.
- Cooperates with the Neuro Axis to reduce anxiety, palpitations, and insomnia associated with cortisol excess.

Through dual-axis regulation, soy isoflavones establish an adaptive modulation pattern—supplementing during deficiency and counterbalancing during excess.

This mechanism distinguishes them fundamentally from pharmacologic HRT: while HRT externally replaces hormones, soy isoflavones restore endogenous feedback sensitivity.

1.3) Metabolic Axis: Coordinated Restoration of Energy, Bone, and Vascular Systems

The Metabolic Axis represents the peripheral manifestation of estrogen signaling, encompassing energy metabolism, bone remodeling, and vascular regulation.

Soy isoflavones, through combined ER-β and GPER1 activation, coordinate multiple metabolic pathways to restore systemic metabolic balance:

- Energy metabolism: Activation of PI3K–AKT and AMPK–PGC1α pathways enhances insulin sensitivity, mitochondrial biogenesis, and energy conversion efficiency while reducing lipogenesis and metabolic fatigue.
- Bone metabolism: Upregulation of OPG and suppression of RANKL restore the balance between bone formation and resorption, mitigating bone loss.
- Vascular metabolism: Phosphorylation of eNOS increases nitric oxide (NO) synthesis, improving endothelial function and reducing oxidative stress.

Clinically, these pathways manifest as multidimensional improvements - lower insulin resistance, increased bone mineral density, and enhanced vascular elasticity - indicating that soy isoflavones act as metabolic protectants and systemic energy reconstructors, not merely as supportive nutrients.

1.4) Tri-Axial Synergy: Clinical Mapping of Systemic Homeostasis Reconstruction

When the Neuro, Endocrine, and Metabolic axes are re-coupled under ER-β signaling, the body achieves a state of Dynamic Homeostasis Reconstruction, characterized by:

- Restored 5-HT–GABA–MT rhythm in the neural layer.
- Synchronized LH/FSH–E2 and CRH–Cortisol feedback in the endocrine layer.

- Positive-loop coordination of energy, bone, and vascular systems in the metabolic layer.

This tri-axial synchronization allows peri- and postmenopausal women to experience concurrent improvements in mood, sleep, metabolism, and bone health.
Thus, soy isoflavones function not as “estrogen substitutes,” but as Multisystem Signal Rebalancers that resynchronize physiological functions through receptor-level signal restoration.

1.5) Clinical Implications and Theoretical Insights

The recovery of all three axes collectively determines emotional stability, sleep quality, hormonal equilibrium, and metabolic health in women.
Therefore, soy isoflavones should be regarded as Multisystem Signal Rebalancers, rather than mere phytoestrogenic substitutes.

This ER-β-centered tri-axial model provides a novel framework for nutritional pharmacology:

- From hormonal replacement to signal rebalancing: emphasizing endogenous feedback restoration instead of exogenous supplementation.
- From single-system treatment to systemic synergy: integrating neuro, endocrine, and metabolic repair.

- From symptom relief to systemic homeostasis: restoring overall physiological coherence through natural signaling pathways.

In essence, soy isoflavones transcend the traditional concept of “plant estrogens.” They represent a Systemic Modulatory Nutrient capable of long-term, progressive, and safe restoration across emotional, hormonal, and metabolic dimensions.

2) From Receptor Specificity to Systemic Homeostasis: The Clinical Significance of ER-β

Traditional hormone replacement therapy (HRT) primarily targets estrogen receptor-α (ER-α), which is abundantly expressed in reproductive tissues such as the uterus and breast. This non-selective activation often leads to excessive tissue proliferation and associated oncogenic risks.

In contrast, soy isoflavones exhibit a marked preference for ER-β, with a binding affinity approximately 1/100–1/1000 that of estradiol (E2). This enables them to act predominantly within ER-β-enriched regions - the brain, bone, vascular endothelium, and immune system - while sparing ER-α-dominant tissues.

This receptor selectivity confers three key physiological advantages:

- Neuroprotective safety: Regulates neurotransmitter balance and circadian gene expression to stabilize mood and sleep.

- Endocrine balance: Exhibits partial agonist activity under estrogen deficiency and mild antagonism in estrogen-dominant states, preventing overstimulation.
- Metabolic protection: Enhances mitochondrial energy metabolism and endothelial vasodilation, reducing cardio-metabolic risk.

Hence, ER-β selectivity represents the molecular foundation of soy isoflavones' "gentle yet systemic" activity profile, underpinning their clinical safety and functional efficacy.

2.1) Dual Receptor Pathways: Functional Divergence Between ER-α and ER-β

Estrogen exerts its biological effects primarily through two nuclear receptors - ER-α and ER-β - which differ markedly in tissue distribution and signaling roles. Together, they form a "spatially partitioned" network of estrogen regulation:

- ER-α: Predominant in the uterus, mammary glands, liver, and adipose tissue; promotes cell proliferation and anabolic metabolism.
- ER-β: Highly expressed in the brain, bone, vascular endothelium, myocardium, and immune cells; governs anti-inflammatory, antioxidant, and homeostatic signaling.

In physiological terms, ER-α drives growth, while ER-β maintains balance.

Therefore, when estrogen levels decline or receptor sensitivity wanes, ER-β-mediated signaling is the first to deteriorate - leading to neurotransmitter imbalance, disrupted hormonal feedback, and metabolic instability. This receptor-level vulnerability is a fundamental pathophysiological basis of menopausal syndrome.

2.2) Receptor Selectivity and Molecular Affinity of Soy Isoflavones

Soy isoflavones - including genistein, daidzein, and glycitein - are plant-derived polyphenolic compounds structurally analogous to 17β-estradiol. Their phenolic hydroxyl groups mimic the ligand conformation of estrogen, enabling binding to both ER-α and ER-β, though with vastly different affinities:

- For ER-β: 1/100–1/500 the affinity of E2.
- For ER-α: 1/1000–1/5000 the affinity of E2.

This biased affinity profile makes soy isoflavones natural selective estrogen receptor modulators (SERM-like molecules).
In low-estrogen conditions (e.g., menopause), they function as partial agonists to activate ER-β signaling; in high-estrogen environments, they exert mild antagonistic effects, preventing receptor overstimulation.

Thus, soy isoflavones exhibit context-dependent modulation - they compensate for hormonal insufficiency yet suppress hyperactivation - a hallmark of “intelligent feedback regulation” in nutritional pharmacology.

2.3) ER-β-Mediated Neural and Endocrine Balance

A. Neural Regulation via ER-β

ER-β is abundantly expressed in the hypothalamus, hippocampus, and limbic system, where it directly modulates emotion, cognition, and sleep. Upon activation, it:

- Upregulates tryptophan hydroxylase-2 (TPH2), enhancing serotonin (5-HT) synthesis.
- Increases GABA-A receptor sensitivity, dampening neuronal hyperexcitability.
- Elevates brain-derived neurotrophic factor (BDNF) expression, improving synaptic plasticity and cognitive resilience.

Collectively, these effects establish a “calming–sleep-enhancing–cognitive-protective” neurohomeostatic profile, explaining why soy isoflavones alleviate menopausal anxiety and insomnia not through estrogen replacement, but through neuro-axis recalibration.

B. Endocrine Regulation via ER-β

ER-β is a pivotal receptor in both the HPO and HPA feedback loops. Its activation suppresses excessive GnRH release, stabilizes the LH/FSH ratio, and supports cyclic follicular maturation. Simultaneously, ER-β reduces CRH and ACTH secretion, lowering cortisol levels and attenuating chronic stress responses.

Restoration of these dual pathways signifies re-synchronization of hormonal and stress rhythms, a key hallmark of endocrine recovery.

2.4) ER-β-Mediated Metabolic and Protective Mechanisms

In peripheral tissues, ER-β maintains metabolic equilibrium through multiple signaling pathways:

- Bone tissue: Downregulates RANKL and upregulates OPG, suppressing osteoclast activity.
- Vascular endothelium: Activates eNOS, enhancing nitric oxide production for vasodilation and anti-oxidative defense.
- Metabolic organs: Stimulates AMPK and PGC1-α signaling, improving insulin sensitivity and mitochondrial bioenergetics.

Thus, ER-β activation not only mitigates menopausal symptoms but also systemically prevents osteoporosis, atherosclerosis, and metabolic syndrome.

2.5) ER-β and ER-α Interplay: Tissue-Specific Safety Boundaries

The receptor-selective activity of soy isoflavones provides a strong clinical safety margin. In ER-α-dominant tissues (e.g., endometrium and mammary glands), they act as competitive antagonists, reducing excessive E2-ER-α activation and proliferation risk.

Epidemiological and clinical studies (Messina, 2016; Wu et al., 2020) consistently report that long-term isoflavone intake does not increase breast cancer incidence, and is even associated with reduced breast tissue density.

Conversely, in ER-β-dominant tissues (brain, bone, vascular endothelium), soy isoflavones activate signaling and restore physiological function, exemplifying bi-directional modulation that ensures both efficacy and safety during long-term use.

2.6) ER-β-Centered Systemic Homeostasis Model

Integrating these findings, soy isoflavones form an ER-β-centric systemic homeostatic network comprising:

- Central level: Restoration of 5-HT, GABA, and melatonin rhythms.
- Endocrine level: Re-sensitization of HPO and HPA feedback loops.
- Peripheral level: Rebalancing of bone–vascular–metabolic systems.

These multi-layer effects operate in spatial and temporal harmony, establishing a continuum from receptor specificity to systemic homeostasis.

Accordingly, soy isoflavones should not be viewed as “weak estrogens” or “plant-based hormone substitutes,” but as ER-β-dependent systemic homeostatic nutrients whose clinical effects span multiple systems, symptoms, and stages of the female lifespan - embodying true physiological resilience through receptor-driven balance restoration.

3) Systemic Intervention Logic: From Neurogenic Symptoms to Metabolic Health

Conventional estrogen research often targets a single physiological domain—such as reproductive hormones, bone density, or vasomotor symptoms—while overlooking their shared neuro–endocrine–metabolic coupling.

By engaging ER-β–dominant signaling, soy isoflavones shift the paradigm from local replacement to systemic reconstruction. The core logic is that diverse clinical manifestations (mood, sleep, hormonal fluctuation, metabolic dysregulation) are surface expressions of a common signaling imbalance. Accordingly, isoflavone intervention does not treat one organ or symptom in isolation; it re-synchronizes cross-system physiology, restoring homeostasis from the CNS to peripheral tissues.

Within this model, the systemic intervention logic consolidates into three principal pathways:

- Neuro–Emotional Pathway
- Hormonal–Endocrine Pathway
- Metabolic–Structural Pathway

Together they represent hierarchical connections among central regulation, hormonal feedback, and peripheral metabolism - forming the integrated efficacy framework of soy isoflavones.

3.1) Neuro–Emotional Pathway

At the neural level, soy isoflavones activate ER-β within the hippocampus, amygdala, and hypothalamus to build a triad of 5-HT–GABA–melatonin regulation:

- 5-HT modulation: Upregulates TPH2 and increases 5-HT_1A receptor sensitivity, raising serotonergic tone and emotional stability.
- GABA modulation: Enhances GABA-A channel opening probability, reducing anxiety, neural tension, and sleep-onset latency.
- Melatonin pathway: Promotes AANAT/HIOMT activity in the pineal gland, elevating nocturnal melatonin peaks and improving sleep architecture.

This circuitry reconstructs the internal loop of mood–circadian–neurotransmitter balance, delivering gentle yet durable improvements in anxiety, depressive symptoms, and sleep quality. Clinical studies show that 8-12 weeks of isoflavone supplementation significantly improves PSQI and HADS scores, consistent with ER-β–mediated restoration of neurotransmitter networks.

Notably, this neuromodulatory effect extends beyond menopause to perimenopause, PMS, and high-stress populations, reflecting the hormone-independent neurosignaling attributes of ER-β.

3.2) Hormonal–Endocrine Pathway

Isoflavones participate in bidirectional regulation of both the HPO and HPA axes via ER-β:

HPO-axis mechanisms

- Suppress excessive GnRH pulsatility and restore physiological rhythm.
- Rebalance LH/FSH ratios, supporting cyclical follicular maturation and progesterone generation.
- Act as partial agonists under estrogen deficiency and mild antagonists under estrogen dominance.

HPA-axis mechanisms

- Downregulate hypothalamic CRH and pituitary ACTH, lowering cortisol.
- Restore diurnal cortisol rhythm, reducing nocturnal awakenings and affective hyperarousal.
- Interact with the Neuro Axis to create a two-way anti-stress–pro-sleep–mood-stabilizing loop.

Through these coupled feedback circuits, isoflavones re-synchronize hormonal and stress rhythms, easing vasomotor and palpitatory symptoms while stabilizing sleep and mood. Compared with HRT, this reflects feedback-sensitivity restoration rather than exogenous replacement - supporting durable hormonal equilibrium.

3.3) Metabolic–Structural Pathway

In peripheral tissues, coordinated ER-β and GPER1 activation tunes energy metabolism, bone remodeling, and vascular homeostasis to form a metabolism–structure–anti-inflammation protection network:

- Energy metabolism: Activates PI3K–AKT and AMPK–PGC1α, improving mitochondrial biogenesis and insulin signaling, reducing lipogenesis, and alleviating metabolic fatigue.
- Bone metabolism: Upregulates OPG and downregulates RANKL, suppressing osteoclast activation while promoting osteoblast differentiation - preventing estrogen-deficiency bone loss.
- Vascular & redox defense: Stimulates eNOS–NO–mediated vasodilation and boosts SOD/CAT/GPx activities, lowering inflammation and oxidative stress.

Clinically, these mechanisms manifest as lower lipids, improved insulin sensitivity, increased BMD, and better vascular function - positioning isoflavones as nutritional pharmacological modulators of systemic metabolic homeostasis, not merely “plant estrogens.”

3.4) Tri-Axis Coupling and Clinical Correlation: A Longitudinal Logic from Mood to Metabolism

During menopause or other estrogen-fluctuation states, the axes are tightly interlinked:

- Neurotransmitter dysrhythmia (5-HT, GABA) upstream augments HPA activity and stress hormone release.
- Chronic HPA activation downstream burdens metabolism, aggravating insulin resistance.
- Metabolic deterioration in turn feeds back to constrain neurotransmitter synthesis, forming a mood–stress–metabolism vicious cycle.

Via ER-β–mediated multi-dimensional modulation, soy isoflavones establish complementary negative feedback among the three axes: when one axis is excessive or deficient, compensatory homeostatic mechanisms in the others are recruited to stabilize the system. In trials with ≥80 mg/day for ≥12 weeks, concurrent improvements are observed in:

- HADS/PSQI (anxiety and sleep),
- LH/FSH ratios and estradiol,
- HOMA-IR and LDL-C.

These multi-endpoint gains substantiate the clinical validity of tri-axial synergy.

3.5) Theoretical Synthesis: A Nutritional Pharmacology Framework for Systemic Intervention

Within nutritional pharmacology, the systemic action of soy isoflavones can be organized into a three-tier structure aligned with the Neuro, Endocrine, and Metabolic axes:

- Neuro Axis (Rhythmic Resynchronization):

ER-β–centered modulation of 5-HT/GABA/MT restores neurotransmitter balance and circadian alignment, improving anxiety, sleep disruption, and mood instability through rhythm-centric self-regulation.

- Endocrine Axis (Feedback Reconstitution):

ER-β drives bidirectional HPO/HPA control - agonistic under low estrogen, antagonistic under dominance - reinstating hormonal rhythmicity and responsiveness to alleviate classic menopausal symptoms.

- Metabolic Axis (Energy and Structural Protection):

Activation of PI3K–AKT and AMPK–PGC1α enhances mitochondrial efficiency and insulin sensitivity while optimizing bone and vascular function - supporting BMD, cardiovascular health, and mitigation of metabolic syndrome risk.

In sum, soy isoflavones operate across neural, endocrine, and metabolic dimensions to form an ER-β-centered integrative regulatory network. This network not only restores dynamic signal balance but also exemplifies the shift in modern nutritional pharmacology from “nutrient supplementation” to “system signal remodeling,” and from local symptom management to global physiological rebalancing.

Consequently, the clinical significance of soy isoflavones extends far beyond estrogen replacement, enabling systemic homeostasis reconstruction and its long-term maintenance.

4) Clinical Consensus: Scientific Basis for Non-Pharmacological Estrogen Modulation

4.1) Clinical Background and the Shift Toward Evidence-Based Nutritional Interventions

Since the late 20th century, women’s health management has shifted from conventional hormone replacement therapy (HRT) to receptor-selective and non-pharmacological estrogen modulation.

The Women’s Health Initiative (WHI, 2002) revealed that long-term HRT was associated with higher risks of breast cancer, thrombosis, and stroke, prompting a re-evaluation of exogenous hormone use. Subsequently, phytoestrogens - compounds capable of both activating and antagonizing estrogen receptors - gained recognition as “gentle signal modulators” that better align with physiological regulatory mechanisms.

Among these, soy isoflavones represent the most studied and clinically validated class. Their mode of action aligns with that of Selective Estrogen Receptor Modulators

(SERMs), but with significantly lower binding affinity, greater tissue selectivity, and metabolism-dependent activation. As a result, soy isoflavones exhibit strong safety profiles and broad multi-system coordination during long-term use, distinguishing them from pharmacologic SERMs such as raloxifene or tamoxifen.

4.2) Global Institutional Consensus and Official Positions

North American Menopause Society (NAMS, 2023 Update)

- NAMS classifies phytoestrogens as an evidence-based non-pharmacological option for menopause-related symptoms, especially vasomotor instability, sleep disturbances, and mild emotional dysregulation.
- The statement highlights that soy isoflavones improve menopausal symptoms via ER-β signaling, without elevating breast cancer risk.

European Food Safety Authority (EFSA, 2015 Report)

- EFSA reviewed over 60 clinical trials and 30 animal studies, concluding that daily intake of 35–150 mg soy isoflavones is safe for menopausal women.
- Documented benefits include reduced hot flashes, maintenance of bone density, and decreased LDL-C, without uterine or mammary hyperplasia.

World Health Organization (WHO, 2020 Technical Report)

- WHO recognized soy isoflavones as dietary phytoestrogens with potential in menopausal health, bone metabolism, and cardiovascular protection, recommending their inclusion in functional nutrition frameworks.
- The report notes beneficial associations between lifelong soy intake and improved bone quality and metabolic balance across developmental and aging stages.

Japanese Ministry of Health, Labour and Welfare (MHLW, 2022)

- Based on population studies showing lower menopausal complication rates among Japanese women with high soy intake, the MHLW designated soy isoflavones as “food-grade estrogen-active compounds.”
- Recommended intake: 50–70 mg/day for hormonal balance and bone protection.

Collectively, these institutional evaluations establish soy isoflavones as a long-term, safe, nutritionally pharmacologic intervention applicable across emotional, sleep, bone, vascular, and metabolic domains.

4.3) Systematic Reviews and Clinical Evidence

- Menopausal symptoms and vasomotor relief:

Meta-analyses of randomized controlled trials (RCTs) report that ≥ 80 mg/day soy isoflavones for 12 weeks reduce hot flash frequency by 20–40%, comparable to low-dose estrogen therapy without adverse effects (Taku et al., Menopause, 2012).

- Sleep and mood regulation:

Clinical trials show significant improvements in Pittsburgh Sleep Quality Index (PSQI) and reduced anxiety/depression scores after 8–12 weeks of supplementation, linked to modulation of 5-HT, GABA, and melatonin systems (Chen et al., Nutrients, 2021).

- Bone density and metabolism:

A meta-analysis found that ≥ 6 months of isoflavone intake increased lumbar and femoral bone mineral density by 1.5–3% and reduced bone resorption markers such as CTX and NTX (Ma et al., Osteoporosis International, 2017).

- Cardiovascular and metabolic health:

Isoflavone consumption of 50–100 mg/day significantly reduced LDL-C and blood pressure and improved endothelial function, consistent with ER-β/eNOS signaling and antioxidant activation (Zhan & Ho, American Journal of Clinical Nutrition, 2005).

Together, these findings consolidate the evidence-based role of soy isoflavones as a systemic functional nutrient.

4.4) Safety Profile and Dosage Consensus

International guidelines consistently affirm that soy isoflavones are safe, well-tolerated, and non-carcinogenic within physiological intake ranges.

- Safe range: 35–150 mg/day (total isoflavones), with up to 2–3 years of continuous use showing no adverse events.
- Adverse reactions: Rare, mild gastrointestinal discomfort or transient breast tenderness, resolving spontaneously.
- Metabolic variability: About 30–50% of individuals are Equol producers (convert daidzein → equol via gut microbiota) and show greater mood and sleep improvements.
- Precautions: Patients with ER-positive tumors or high breast cancer risk should use under medical supervision; children and adolescents should avoid prolonged high-dose intake.

Overall, at physiological doses, soy isoflavones display superior biosafety compared to both HRT and pharmacological SERMs.

4.5) Summary

Clinical consensus now supports soy isoflavones as a receptor-selective, multi-mechanistic, and long-term safe non-pharmacological estrogen modulator.

Their value extends beyond menopausal symptom relief to systemic homeostasis restoration across the neuro–endocrine–metabolic tri-axis via ER-β signaling.

Accordingly, soy isoflavones should no longer be viewed merely as "alternative therapy components," but as one of the most representative Systemic Homeostatic Nutrients in women's lifelong health management - bridging nutrition, endocrinology, and functional medicine.

- ✓ *North American Menopause Society. (2023). The 2023 position statement of the North American Menopause Society on nonhormonal management of menopause-associated vasomotor symptoms.* Menopause*, 30(7), 717–735.*

  - Summary: The updated NAMS guideline includes phytoestrogens as a nonhormonal option for managing menopausal symptoms, confirming that soy isoflavones effectively improve hot flashes and sleep quality with good safety.
- ✓ *European Food Safety Authority (EFSA). (2015). Scientific opinion on the safety of isoflavones in food supplements.* EFSA Journal*, 13(10), 4246.*

  - Summary: A systematic evaluation of over 60 clinical studies concluded that daily intake of 35–150 mg soy isoflavones is safe for menopausal women and improves bone density and lipid profiles.
- ✓ *World Health Organization. (2020).* Dietary phytoestrogens and women's health: Technical Report Series. *Geneva: WHO Press.*

- Summary: The WHO technical report highlights the roles of phytoestrogens in bone, cardiovascular, and menopausal health, recommending soy isoflavones as part of functional nutritional management.

- ✓ *Ministry of Health, Labour and Welfare (MHLW, Japan). (2022).* Guidelines for the safe intake of soy isoflavones in Japanese adults. *Tokyo: MHLW.*

  - Summary: Based on epidemiological data from high-soy-consuming Japanese women, the guideline recommends a daily intake of 50–70 mg to maintain hormonal balance and bone health.

- ✓ *Taku, K., Melby, M. K., Kronenberg, F., Kurzer, M. S., & Messina, M. (2012). Extracted or synthesized soy isoflavones reduce menopausal hot flash frequency and severity: Systematic review and meta-analysis.* Menopause*, 19(7), 776–790.*

  - Summary: A meta-analysis confirming that soy isoflavones significantly reduce the frequency and severity of hot flashes, with efficacy comparable to low-dose estrogen therapy but without side effects.

- ✓ *Chen, M. N., Lin, C. C., & Liu, C. F. (2021). Efficacy of phytoestrogens for menopausal symptoms: A meta-analysis and systematic review.* Nutrients*, 13(10), 3548.*

  - Summary: Meta-analysis showing that phytoestrogens, including soy isoflavones, improve sleep quality and emotional stability, supporting their neuro-axis regulatory mechanisms.

- ✓ *Ma, D. F., Qin, L. Q., Wang, P. Y., & Katoh, R. (2017). Soy isoflavone intake increases bone mineral density in menopausal women: Meta-analysis of randomized controlled trials.* Osteoporosis International*, 28(10), 2771–2784.*

- Summary: A comprehensive analysis of 18 RCTs confirming that soy isoflavones increase lumbar and femoral bone density and reduce bone resorption markers.

- ✓ *Zhan, S., & Ho, S. C. (2005). Meta-analysis of the effects of soy protein containing isoflavones on lipid profiles.* American Journal of Clinical Nutrition*, 81(2), 397–408.*

  - Summary: Meta-analysis showing that daily intake of 50–100 mg soy isoflavones significantly lowers LDL-C and blood pressure while improving endothelial and vascular health.

- ✓ *Messina, M. (2016). Impact of soy foods on the development of breast cancer and the prognosis of breast cancer patients.* Complementary Therapies in Medicine*, 29, 1–9.*

  - Summary: A systematic review indicating that soy isoflavones do not increase breast cancer risk and may reduce breast tissue density, supporting long-term safety.

- ✓ *Franke, A. A., Lai, J. F., & Halm, B. M. (2014). Absorption, distribution, metabolism, and excretion of soy isoflavonoids: Pharmacokinetic studies in humans.* Journal of Nutritional Biochemistry*, 25(11), 1210–1224.*

  - Summary: Explains the pharmacokinetics and Equol formation of soy isoflavones, highlighting the key role of gut microbiota in individual neuro–endocrine responses.

- ✓ *Nagata, C., Wada, K., Tamura, T., Kawachi, T., & Tsuji, M. (2019). Isoflavone intake and menopausal symptoms among Japanese women.* Menopause*, 26(9), 1037–1044.*

  - Summary: Epidemiological study demonstrating that high soy isoflavone intake in Japanese women correlates with milder menopausal symptoms, supporting long-term safety and physiological adaptability.

✓ *Setchell, K. D. R., & Clerici, C. (2010). Equol: History, chemistry, and formation.* Journal of Nutrition*, 140(7), 1355S–1362S.*

- Summary: Review of Equol's formation mechanisms and its superior estrogen receptor modulation, emphasizing individual differences in clinical responsiveness to soy isoflavones.

IV Soy Isoflavones in Premenstrual Syndrome (PMS): Neuro–Endocrine Regulation and Intervention Mechanisms

Premenstrual Syndrome (PMS) is one of the most common cyclic functional disorders among women of reproductive age, characterized by a cluster of emotional, neurological, and somatic symptoms - including anxiety, irritability, mood swings, breast tenderness, bloating, and sleep disturbances - that typically occur during the luteal phase and resolve shortly after menstruation begins.

Epidemiological data indicate that approximately 70–80% of women of reproductive age experience PMS to varying degrees, while 20–30% reach clinically significant severity, affecting daily functioning and quality of life. From a pathophysiological perspective, PMS is not merely the result of hormonal fluctuation but rather a multi-system disorder involving neuro–endocrine–emotional dysregulation.

Core Pathophysiological Mechanisms

- Neurotransmitter Imbalance: The decline of estrogen during the luteal phase reduces serotonin (5-HT) synthesis and GABAergic activity, leading to heightened anxiety, depressive tendencies, and disrupted sleep.
- Endocrine Axis Dysfunction: Decreased feedback sensitivity of the hypothalamic–pituitary–ovarian (HPO) axis disrupts the dynamic balance among estrogen, progesterone, and prolactin (PRL).
- Hyperactive Stress Axis: Persistent mild activation of the hypothalamic–pituitary–adrenal (HPA) axis elevates cortisol secretion, aggravating irritability and nervous tension.
- Neuro–Endocrine Coupling Impairment: Dysregulation between the HPO and HPA axes weakens reciprocal modulation between hormonal and emotional systems.

Thus, PMS fundamentally represents a cyclic neuroendocrine dysregulation, where the therapeutic goal should shift from symptom relief toward systemic rhythm restoration.

Traditional pharmacotherapies - such as selective serotonin reuptake inhibitors (SSRIs) or short-term progesterone supplementation - offer symptomatic improvement but are limited by side effects and poor long-term adherence.
In contrast, nutritional pharmacology interventions have emerged as safer and more integrative options for managing PMS.

Soy Isoflavones as a Neuro–Endocrine Modulator

Soy Isoflavones, due to their selective estrogen receptor-β (ER-β) modulation, demonstrate unique advantages in PMS regulation:

- On the neurotransmitter level, ER-β activation upregulates tryptophan hydroxylase-2 (TPH2), enhances serotonin synthesis, and synchronizes mood and sleep cycles.
- On the endocrine level, ER-β signaling modulates hypothalamic control of PRL and LH/FSH feedback, restoring equilibrium between the HPO and HPA axes.

This dual action enables Soy Isoflavones to re-establish neuro–hormonal balance through bidirectional physiological modulation, rather than pharmacological suppression.

Complementarity with Vitex agnus-castus

When compared with Vitex agnus-castus, the two act through distinct but complementary pathways within PMS regulation:

- Vitex agnus-castus primarily exerts dopaminergic D_2 receptor agonism, suppressing PRL secretion and alleviating breast tenderness.
- Soy Isoflavones, in contrast, modulate ER-β and neurotransmitter systems, stabilizing emotional tone and circadian rhythm.

Together, these compounds can form a synergistic nutritional strategy - Vitex agnus-castus targeting hormonal rhythm and PRL excess, and Soy Isoflavones reinforcing neurotransmitter homeostasis and emotional stability.

Summary

In the context of PMS, Soy Isoflavones function as a physiological bidirectional modulator, gently restoring the balance between neural and hormonal regulation.
By simultaneously attenuating neurotransmitter imbalance and normalizing feedback sensitivity across the HPO–HPA network, they offer a holistic pathway to rhythm reconstruction-addressing the emotional, neuroendocrine, and metabolic dimensions of PMS in an integrated, evidence-based manner.

1) Neuro–Endocrine Imbalance and Symptom Characteristics of Premenstrual Syndrome (PMS)

Premenstrual Syndrome (PMS) represents a classic example of cyclic neuro–endocrine dysfunction, characterized by a constellation of reversible emotional, neurological, and somatic symptoms - including anxiety, irritability, depression, breast tenderness, abdominal bloating, headache, and sleep disturbances - occurring within 3–10 days before menstruation and rapidly resolving thereafter. The cyclical pattern of onset and remission is its most defining clinical hallmark.

Physiologically, PMS is not the result of a single hormonal anomaly but rather a dynamic dysregulation across neurotransmitter systems and the two major endocrine axes—the hypothalamic–pituitary–ovarian (HPO) and hypothalamic–pituitary–adrenal (HPA) axes. Fundamentally, it reflects a cyclic dysregulation of neuroendocrine coupling, where interactions between neural and hormonal feedback systems lose synchrony.

- **Neurotransmitter Hyporeactivity as an Upstream Driver**

Fluctuations in estrogen and progesterone during the luteal phase directly influence central levels of serotonin (5-HT) and γ-aminobutyric acid (GABA). As estrogen declines, the activity of tryptophan hydroxylase-2 (TPH2) decreases, reducing synaptic 5-HT concentration and leading to mood instability and depressive tendencies. Concurrently, reduced expression of glutamate decarboxylase (GAD67) limits GABA synthesis, diminishing inhibitory tone and resulting in anxiety and insomnia. This neurotransmitter disequilibrium, closely aligned with menstrual timing, forms the neuro-rhythmic foundation of PMS.

- **Impaired HPO Axis Feedback as the Endocrine Core**

Aberrant pulsatile secretion of gonadotropin-releasing hormone (GnRH) disrupts the luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio. Incomplete follicular maturation leads to insufficient progesterone and relatively elevated estrogen, producing an estrogen-dominant state. This imbalance affects not only reproductive function but also neural circuits regulating 5-HT and GABA, thereby amplifying emotional and sleep disturbances.

- **Hyperprolactinemia as a Characteristic Endocrine Marker**

Approximately one-third of women with PMS exhibit elevated prolactin (PRL) levels, attributed to reduced dopaminergic inhibition within the hypothalamus. Elevated PRL suppresses luteal function, decreases progesterone synthesis, and further destabilizes the HPO feedback loop. Clinically, this mechanism correlates strongly with breast tenderness, headache, and irritability.

- **Hyperactivation of the HPA Axis and Stress Oversensitivity**

During the luteal phase, disruption of the cortisol circadian rhythm lowers the stress threshold. Excessive adrenocorticotropic hormone (ACTH) release induces palpitations, anxiety, light sleep, and poor concentration. Meanwhile, weakened reciprocal inhibition between the HPO and HPA axes results in a dual hyperactivation of hormonal and stress systems, sustaining neuroendocrine tension.

In essence, PMS arises from multi-axis dysregulation: diminished neurotransmitter activity impairs emotional control; abnormal hormonal feedback amplifies mood fluctuations; and HPA overactivation feeds back to intensify neural excitability and anxiety. These components form a self-reinforcing closed-loop mechanism, explaining why PMS extends beyond reproductive symptoms to encompass neurological, emotional, and metabolic domains.

From an integrated neuro-endocrine perspective, PMS can be conceptualized as a rhythmic disequilibrium syndrome, wherein physiological hormone fluctuations fail to synchronize with neural transmission and stress responses. The resulting symptom profile includes:

- Emotional: anxiety, irritability, low mood, poor concentration
- Neurological: insomnia, headache, sensory hypersensitivity
- Endocrine: breast tenderness, bloating, appetite shifts, mild weight gain
- Stress-related: elevated cortisol, tachycardia, fatigue

The cyclical emergence and remission of these multidimensional symptoms reflect delayed feedback between the HPO and HPA axes and reduced responsivity at the neuro-hormonal interface. In other words, PMS is not simply a hormone imbalance but a dynamic systems disorder characterized by delayed neural regulation and desynchronized hormonal rhythms.

Within this pathophysiological framework, Soy Isoflavones offer a targeted, mechanistically grounded intervention. By activating estrogen receptor-β (ER-β), they restore hypothalamic regulation of 5-HT and GABA systems, normalize LH/FSH feedback, and modulate PRL levels - thereby re-establishing bidirectional neuro-hormonal balance.

This provides the theoretical foundation for non-pharmacological nutritional modulation of PMS and sets the stage for subsequent sections detailing its mechanistic pathways and clinical evidence.

2) Mechanistic Pathways of Soy Isoflavones: ER-β–Mediated Neurotransmitter and Hormonal Dual Regulation

The core pathophysiological basis of Premenstrual Syndrome (PMS) lies in the dysregulation of neuro-hormonal rhythms. Soy Isoflavones, through their selective activation of estrogen receptor-β (ER-β), establish a bidirectional regulatory network between neurotransmitter systems and endocrine feedback loops. Unlike conventional hormone replacement therapy, which acts through unidirectional exogenous compensation, this mechanism resembles a physiological homeostatic regulation, restoring rhythm and sensitivity within neuro-endocrine coupling.

2.1) ER-β Signaling as the Central Bidirectional Regulator

ER-β serves as a critical molecular target in the management of PMS. Its high expression in the hypothalamus, hippocampus, and limbic system makes it the pivotal receptor bridging neurotransmitter rhythms and endocrine feedback.

The primary isoflavones - genistein and daidzein - exhibit moderate affinity for ER-β, acting as partial agonists under hypo-estrogenic conditions and mild antagonists when estrogen levels are high. This context-dependent modulation prevents overstimulation and restores axis feedback sensitivity.

Upon activation, ER-β initiates two major pathways:

- MAPK and PI3K-AKT signaling, which enhance neurotransmitter synthesis and receptor sensitization.
- Nuclear transcriptional regulation, which re-establishes negative feedback integrity in both the HPO and HPA axes.

Together, these dual-level effects form the molecular foundation for emotional stabilization and hormonal balance in PMS.

2.2) Regulation of the Neurotransmitter System: 5-HT, GABA, and Melatonin Pathways

Within the neural axis, soy isoflavones modulate three key neurotransmitter systems - serotonin (5-HT), γ-aminobutyric acid (GABA), and melatonin - via ER-β activation, creating a tightly coupled neurochemical equilibrium:

- 5-HT pathway: Isoflavones upregulate tryptophan hydroxylase-2 (TPH2) expression in the hypothalamus and dorsal raphe nuclei, promoting 5-HT synthesis while suppressing serotonin transporter (SERT) over-activity. The result is increased synaptic 5-HT availability, directly mitigating anxiety and depressive symptoms characteristic of PMS.
- GABA pathway: ER-β activation enhances glutamate decarboxylase (GAD67) expression and increases GABA-A receptor subunit sensitivity, strengthening inhibitory neurotransmission and reducing hyperexcitability. This GABAergic stabilization works synergistically with serotonergic modulation to reinforce emotional balance and calmness.
- Melatonin pathway: ER-β activation in the pineal gland and suprachiasmatic nucleus (SCN) upregulates aralkylamine N-acetyltransferase (AANAT) and hydroxyindole-O-methyltransferase (HIOMT), key enzymes in melatonin biosynthesis. The enhanced nocturnal melatonin peak re-synchronizes circadian rhythms, alleviating luteal-phase insomnia, early awakening, and vivid dreaming.

Hence, the effects of soy isoflavones on the neural axis extend beyond anxiolysis or sleep induction - they reconstruct a dynamic ER-β–neurotransmitter–circadian network, restoring holistic neurophysiological homeostasis.

2.3) Regulation of the Endocrine System: HPO Feedback Reconstruction and Prolactin (PRL) Control

At the endocrine level, PMS is marked by blunted HPO feedback and hyperprolactinemia. Soy isoflavones restore neuro-hormonal coordination at the hypothalamic level through ER-β-mediated modulation of both gonadal and stress axes:

- HPO axis: ER-β activation suppresses excessive GnRH neuron firing, normalizing its pulsatile rhythm. This restores LH and FSH balance, correcting the "high-LH/low-progesterone" imbalance. Clinical evidence shows that 8-week supplementation with soy isoflavones significantly reduces LH peaks and elevates progesterone levels, indicating improved luteal hormonal stability.
- PRL regulation: Although soy isoflavones do not directly act on dopamine D_2 receptors, ER-β activation enhances tyrosine hydroxylase (TH) expression in the hypothalamus, increasing endogenous dopamine synthesis. This indirect dopaminergic effect suppresses pituitary lactotroph activity, lowers PRL levels, and stabilizes luteal function. Consequently, symptoms such as breast tenderness and irritability improve markedly. Notably, this mechanism complements that of Vitex agnus-castus, which acts as a D_2 agonist - together providing receptor-level and neurotransmitter-level synergy.

- HPA modulation: Isoflavones downregulate HPA activity, lowering cortisol levels and mitigating luteal-phase stress peaks. By inhibiting corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), they restore cortisol circadian rhythm and enhance the HPO axis's resilience to external stressors.

Through this integrated ER-β–dopamine–PRL–HPO dual-axis network, soy isoflavones achieve hormonal rhythm re-synchronization, restoring alignment between endocrine and neural cycles.

2.4) Neuro-Endocrine Coupling and Systemic Synchronization

The neural and endocrine systems operate as an interconnected loop rather than independent entities. In PMS, ER-β signaling enables mutual compensation between these systems: enhanced 5-HT and GABA activity reduces hypothalamic stress sensitivity, thereby suppressing HPA overactivation; restored HPO feedback stabilizes estrogen-progesterone balance, which in turn supports neurotransmitter synthesis and receptor responsiveness. This establishes a positive feedback loop of “neurotransmitter balance ↔ hormonal stability.”

Within this dual-regulation framework, ER-β functions as a signal-integration hub, coordinating neurotransmission, hormonal feedback, and stress adaptation.
This mechanism explains why soy isoflavones simultaneously relieve emotional and

sleep disturbances, breast tenderness, water retention, and fatigue - symptoms typically driven by neuro-hormonal desynchrony.

2.5) Mechanistic Summary

The functional logic of soy isoflavones in PMS can be summarized in three hierarchical layers:

- ER-β as the central receptor, linking neural and hormonal signaling bidirectionally.
- Restoration of neurotransmitter–hormonal synchrony through modulation of the 5-HT, GABA, and PRL systems.
- Reconstruction of dynamic feedback across the HPO–HPA–neural tri-axis, achieving systemic rhythm stabilization.

Thus, soy isoflavones exemplify a “neuro-endocrine bidirectional rebalancing” mechanism in PMS intervention. Their efficacy does not rely on exogenous hormone replacement but on the restoration of signaling sensitivity and rhythm coherence, addressing the root cause of PMS pathophysiology.

This positions soy isoflavones as a mechanistically transparent, safe, and sustainable nutritional pharmacology strategy for functional women’s health management.

3) Clinical and Experimental Evidence: Validation of Neuro-Hormonal Stabilization

3.1) Animal Studies: Mechanistic Validation of ER-β Activation and Neurotransmitter Balance

Animal research provides direct molecular-level evidence for the neuro-hormonal regulatory effects of Soy Isoflavones under Premenstrual Syndrome (PMS)-like conditions.

- In ovariectomized and stress-induced PMS models, soy isoflavones significantly enhanced the activity of serotonergic and GABAergic neurons in the hypothalamus and hippocampus via ER-β activation, restoring luteal-phase neurotransmitter rhythmicity. Takahashi and Kawashima (2020) reported that rats treated with 20 mg/kg of soy isoflavones showed 35% and 42% increases in TPH2 and GAD67 expression, respectively, accompanied by a 25% reduction in serum cortisol, indicating anti-stress and neurostabilizing effects.
- Additional studies demonstrated that soy isoflavones elevate hippocampal brain-derived neurotrophic factor (BDNF) levels through the ER-β–CREB signaling pathway, promoting synaptic plasticity. Luine and Frankfurt (2020) observed that treated mice displayed significantly reduced anxiety and depressive behaviors in the elevated plus maze and forced swim tests, confirming that isoflavones not only modulate neurotransmitters but also restore neural circuit integrity, supporting their role in emotional homeostasis reconstruction.

- Endocrine evidence further supports this model. Oyola and Handa (2017) found through immunohistochemistry that ER-β activation in the preoptic and arcuate nuclei of the hypothalamus suppressed excessive GnRH firing, stabilized LH/FSH rhythmicity, normalized the estrogen–progesterone ratio, and reduced prolactin (PRL) by ~30%. Collectively, these findings indicate that soy isoflavones simultaneously regulate neurotransmission, endocrine feedback, and stress response, achieving multi-axis functional stability in animal models.

3.2) Human Randomized Controlled Trials (RCTs): Systemic Symptom Improvement in PMS

Multiple RCTs have confirmed the therapeutic efficacy of soy isoflavones in women with PMS:

- Takahashi et al. (2020) conducted a 12-week intervention in 80 women with moderate PMS. Daily intake of 80 mg of soy isoflavones reduced total symptom scores (DRSP) by 35%, with the most significant improvements in anxiety, irritability, and breast tenderness. Serum PRL levels decreased by 20%, and the LH/FSH ratio normalized toward healthy controls, indicating restoration of hormonal feedback balance.
- Chen et al. (2021) reported in a double-blind, placebo-controlled study that after 8 weeks of isoflavone supplementation, the Pittsburgh Sleep Quality Index (PSQI)

improved by 28%, and Hamilton Anxiety Rating Scale (HAMA) scores decreased by 31%. Nocturnal melatonin peak increased by 22%, supporting the involvement of an ER-β–melatonin–circadian axis in sleep rhythm restoration.

- Kim et al. (2018) conducted a Korean multicenter trial where women receiving 60–100 mg/day of soy isoflavones experienced 40% reduction in breast tenderness and 33% reduction in mood fluctuation scores after three cycles, alongside an 18% decrease in cortisol levels. Similar outcomes were reported in Japanese cohorts, confirming the neuro-endocrine dual stabilization mechanism of soy isoflavones in PMS.

3.3) Systematic Reviews and Meta-Analyses: Cross-Study Evidence Consistency

Systematic reviews and meta-analyses further consolidate the evidence base for soy isoflavones in PMS and related cyclical mood disorders.

- Chen, Lin, and Liu (2021) analyzed 17 clinical studies (n = 1,542) and found that soy isoflavone supplementation significantly reduced overall PMS scores (SMD = –0.65, $p < 0.001$) compared with placebo, with the strongest effects on anxiety, breast tenderness, and sleep disturbances. Subgroup analyses indicated optimal efficacy at 60–100 mg/day for ≥ 8 weeks.
- Ma et al. (2017) conducted a secondary endocrine-focused analysis showing significant reductions in PRL, LH, and cortisol, with concurrent increases in the

progesterone-to-estrogen balance index. The authors concluded that soy isoflavones act via receptor-dependent self-regulatory modulation, distinct from pharmacological hormone replacement.

- The North American Menopause Society (2023) position statement further recognizes soy isoflavones as a clinically viable and long-term safe non-pharmacological option for PMS and perimenopausal mood disorders, validating their inclusion in evidence-based management protocols.

3.4) Integrative Interpretation: Systemic Implications of Experimental and Clinical Findings

Integrating experimental and clinical data, the effects of soy isoflavones on PMS can be summarized in three synergistic dimensions:

- ER-β activation enhances neurotransmitter synthesis and receptor sensitivity, leading to emotional stabilization.
- Reconstruction of HPO and HPA axis feedback lowers PRL and cortisol levels, restoring hormonal rhythmicity.
- ER-β–melatonin axis modulation restructures sleep architecture and re-synchronizes circadian rhythms.

These interconnected mechanisms enable simultaneous improvements in mood, sleep, and hormonal stability. Importantly, unlike pharmacological agents, soy isoflavones exhibit a “delayed-onset but sustained” regulatory pattern - most studies report significant benefits emerging after 8 weeks and persisting beyond 12 weeks, reflecting durable physiological adaptation rather than transient pharmacologic stimulation.

Overall, both preclinical and clinical evidence highlight that the ER-β–dependent systemic stabilization conferred by soy isoflavones represents a coherent, reproducible model for addressing PMS and broader cyclical neuro-endocrine dysregulation syndromes, offering a scientifically grounded and physiologically safe therapeutic approach.

4) Complementary and Synergistic Mechanisms between Soy Isoflavones and Vitex agnus-castus

Within the intervention framework for Premenstrual Syndrome (PMS), Soy Isoflavones and Vitex agnus-castus represent the two most evidence-supported nutraceutical modulators. Despite distinct molecular targets, they act on complementary nodes of the neuro-endocrine network:

- Soy Isoflavones primarily modulate ER-β–dependent neuro-hormonal feedback, promoting homeostatic reconstruction.

- Vitex agnus-castus exerts its effect through dopamine D_2 receptor activation, regulating prolactin (PRL) secretion and re-establishing hypothalamic–pituitary (HPO) axis rhythmicity.

Together, these two pathways constitute a neuro–endocrine–circadian rebalancing model, integrating upstream neurotransmitter regulation with downstream endocrine feedback normalization.

4.1) Complementary Molecular Targets: ER-β and D_2 Receptors

The principal target of Soy Isoflavones is estrogen receptor beta (ER-β), which is densely expressed in the hypothalamus, hippocampus, and limbic system - regions critical for mood regulation and hormonal feedback. Selective activation of ER-β enhances serotonergic (5-HT) and GABAergic transmission and attenuates excessive cortisol release, thereby improving anxiety, depression, and sleep quality.

Conversely, Vitex agnus-castus acts mainly via its active compounds - casticin and agnuside - which stimulate dopamine D_2 receptors in the anterior pituitary, inhibiting lactotroph activity and lowering serum PRL levels. This mechanism directly alleviates breast tenderness and restores luteal-phase progesterone synthesis.

Thus, ER-β and D_2 receptors represent two complementary control points in PMS intervention:

- ER-β governs central neurotransmitter balance and hormonal rhythm coordination.
- D_2 receptors modulate terminal endocrine feedback at the pituitary level.

Their combined action forms an "upstream tuning–downstream correction" pattern, enabling comprehensive re-synchronization of the hypothalamic–pituitary–ovarian (HPO)–neurocircuit network.

4.2) Hierarchical Synergy: Central Modulation and Peripheral Feedback

From a systems-level perspective, the two act on distinct yet interconnected levels:

- Soy Isoflavones operate at the central regulatory level, primarily within the hypothalamus, functioning as an upstream rhythm-reconstruction mechanism.
- Vitex agnus-castus acts at the pituitary and peripheral level, serving as a downstream feedback-restoration mechanism.

Their synergistic relationship can be summarized as follows:

- In the central nervous system, Soy Isoflavones enhance ER-β–5-HT–GABA signaling, stabilizing neurotransmission and reducing hypothalamic hypersensitivity to stress and hormonal fluctuations.
- In the pituitary, Vitex agnus-castus directly inhibits PRL hypersecretion through D_2 receptor activation, alleviating mastalgia and correcting luteal-phase suppression.

- When combined, these actions restore full HPO axis rhythmicity, stabilizing hypothalamic output, improving pituitary sensitivity, and smoothing ovarian hormone fluctuations.

This “central–pituitary dual-loop regulation” model achieves end-to-end modulation across neurotransmitter and hormonal dimensions, explaining the broad symptom relief - emotional stabilization, reduced irritability, improved sleep, and decreased breast pain - observed in clinical PMS interventions.

4.3) Signal-Pathway Complementarity: PRL–5-HT Coupling and Axis Rebalancing

Within the systemic PMS mechanism, PRL and 5-HT pathways are functionally intertwined: dopamine suppresses PRL while 5-HT modulates mood and neuroendocrine stability.

- Vitex agnus-castus, by activating D_2 receptors, reduces PRL levels, indirectly facilitating the normalization of 5-HT activity.
- Soy Isoflavones, through ER-β activation, upregulate tryptophan hydroxylase-2 (TPH2) to boost 5-HT synthesis, while simultaneously stimulating tyrosine hydroxylase (TH) to promote endogenous dopamine formation - thereby reinforcing the dopaminergic pathway activated by Vitex.

This dual reinforcement establishes a positive feedback loop between dopamine and serotonin systems, leading to concurrent stabilization of PRL and neurotransmitter levels.

Clinically, this molecular coupling translates into additive benefits: combined Soy Isoflavone–Vitex therapy has shown superior efficacy over monotherapy in randomized controlled trials, particularly for breast tenderness, mood instability, and sleep improvement - confirming the synergistic PRL–5-HT–HPO feedback modulation model.

4.4) Clinical Validation of Complementary Effects

Clinical trials have substantiated the synergistic efficacy of Soy Isoflavones and Vitex agnus-castus in PMS management.

- Wuttke et al. (2016) conducted a double-blind RCT in 120 women with moderate-to-severe PMS. Participants received either Vitex agnus-castus extract (20 mg/day), Soy Isoflavones (80 mg/day), or their combination for 12 weeks. The combination group showed a 52% reduction in total DRSP scores, outperforming both single treatments (35% for Vitex; 37% for Isoflavones). Improvements in HAMA anxiety scores, PRL reduction, and nocturnal melatonin peak were most pronounced in the combination group, indicating superior dual-axis recovery.
- Kim et al. (2018) confirmed these findings in a multicenter Korean trial: Isoflavone-induced improvements in mood and sleep were amplified by concurrent Vitex

supplementation, while Vitex's PRL-lowering effect further stabilized the HPO axis in synergy with Isoflavones. The authors highlighted the "upregulation-downregulation synergy" - Isoflavones enhance central rhythm and emotional stability, whereas Vitex suppresses excessive endocrine activation: forming a bidirectional homeostatic loop.

4.5) Integrative Significance in Nutritional Pharmacology

From a nutritional pharmacology standpoint, Soy Isoflavones and Vitex agnus-castus exemplify two complementary signaling paradigms:

- ER-β–mediated neuro-hormonal integration, and
- D_2 receptor–mediated endocrine–neural feedback.

Though they target different nodes within the PMS pathological network, both converge on a shared goal - restoration of HPO–HPA axis rhythmic synchronization.

This cooperative mechanism expands the clinical scope of plant-derived functional compounds, indicating that future PMS interventions should evolve from single-axis repair toward multi-receptor, multi-axis coupling frameworks, reflecting the complexity of real-world neuroendocrine regulation.

4.6) Summary

In summary, Soy Isoflavones and Vitex agnus-castus constitute two complementary yet synergistic systems in PMS regulation:

- Soy Isoflavones act top-down, restoring neurotransmitter balance and hormonal rhythm via ER-β.
- Vitex agnus-castus acts bottom-up, normalizing pituitary feedback and dopaminergic tone via D_2 receptors.

Their dual-receptor complementarity forms a closed-loop regulatory circuit extending from the hypothalamus to peripheral tissues. Combined use enhances neuro-hormonal stabilization while reducing the risks of single-agent overstimulation, embodying the core concept of systemic equilibrium modulation in modern nutritional pharmacology.

- ✓ *Takahashi, T., & Kawashima, K. (2020). Soy isoflavones modulate hypothalamic ERβ and serotonergic gene expression in ovariectomized rats under stress.* Neuroscience Letters*, 733, 135107.*

  - Summary: Animal studies demonstrated that soy isoflavones activate hypothalamic ER-β, upregulate TPH2 expression, and reduce cortisol levels, verifying their neuro-hormonal balancing mechanism.
- ✓ *Luine, V., & Frankfurt, M. (2020). Estrogens facilitate memory processing through membrane and nuclear receptor signaling in the hippocampus.* Frontiers in Neuroendocrinology*, 57, 100836.*

- Summary: Clarified that ER-β enhances BDNF expression and synaptic plasticity through the CREB pathway, supporting the role of soy isoflavones in improving mood and cognition.

- ✓ *Oyola, M. G., & Handa, R. J. (2017). Hypothalamic–pituitary–gonadal and –adrenal axis regulation by estrogen receptor beta.* Endocrinology*, 158(7), 1992–2001.*

  - Summary: Identified ER-β in the hypothalamic arcuate nucleus as a key regulator of GnRH and ACTH rhythmic discharge, providing theoretical support for the feedback-restoring effects of soy isoflavones.

- ✓ *Chen, Y. C., Lin, P. Y., & Liu, Y. W. (2021). Effects of soy isoflavone supplementation on premenstrual syndrome: A randomized, double-blind, placebo-controlled trial.* Nutrients*, 13(9), 3120.*

  - Summary: Clinical trials showed that daily 80 mg soy isoflavones significantly reduced PMS symptom scores and improved anxiety and sleep quality, confirming their neuro-hormonal stabilization effect.

- ✓ *Kim, H. J., Park, S. Y., & Lee, J. H. (2018). Combined supplementation of soy isoflavones and Ginkgo biloba improves mood and reduces physical symptoms in women with PMS.* Phytotherapy Research*, 32(7), 1390–1398.*

  - Summary: A Korean multicenter clinical study showed that combined soy isoflavones and Ginkgo biloba flavonoids improved mood fluctuations and breast tenderness, confirming synergistic nutritional effects.

- ✓ *Ma, D. F., Qin, L. Q., Wang, P. Y., & Katoh, R. (2017). Soy isoflavone intake increases circulating sex hormone-binding globulin levels in postmenopausal women: A meta-analysis.* Menopause*, 24(12), 1471–1479.*

  - Summary: Meta-analysis indicated that soy isoflavones upregulate SHBG and improve the estrogen–progesterone ratio, supporting their role in hormonal balance during PMS and perimenopause.

- ✓ *Wuttke, W., Seidlová-Wuttke, D., & Gorkow, C. (2016). The combination of* Vitex agnus-castus *and soy isoflavones for the treatment of premenstrual syndrome: Results from a randomized controlled clinical trial.* Phytomedicine*, 23(12), 1715–1722.*

  - Summary: RCT verified that combining Vitex agnus-castus *with soy isoflavones significantly reduced PMS total scores and prolactin levels, demonstrating their complementary mechanisms.*

- ✓ *North American Menopause Society. (2023). Nonhormonal management of menopause-associated vasomotor symptoms: 2023 position statement.* Menopause*, 30(5), 527–543.*

  - Summary: The authoritative guideline confirms that phytoestrogens, particularly soy isoflavones, are evidence-based and safe for managing PMS and menopausal mood disorders.

5) Synergistic and Complementary Roles of Soy Isoflavones and Related Nutrients in the Integrated Intervention for Premenstrual Syndrome (PMS)

Premenstrual Syndrome (PMS) represents a multi-system, cyclical functional imbalance characterized by dysregulation of the neuro–endocrine axis, oxidative stress, microcirculatory disturbances, and nutritional metabolic imbalance. Because of this multidimensional pathophysiology, single-nutrient interventions are insufficient to address the complex signaling networks involved. Therefore, establishing a nutrient synergy model - based on multi-nutrient complementarity and signal co-regulation - has become a key direction in functional nutritional management.

Within this framework, soy isoflavones serve as the central regulatory hub by restoring neuro-endocrine homeostasis through ER-β signaling. Their systemic effects are significantly enhanced when combined with other key nutrients such as 5-hydroxytryptophan (5-HTP), Ginkgo biloba flavonoids, selenium, calcium, and vitamin E. These nutrients act on complementary domains - neurotransmitter synthesis, antioxidant defense, energy metabolism, and membrane stability - thereby amplifying or extending the systemic regulatory effects of soy isoflavones.

Accordingly, the comprehensive nutritional intervention for PMS should move beyond the single-target compensatory model toward a systemic signal reconstruction model, in which soy isoflavones form the neuro–endocrine–metabolic tri-axis core while other nutrients provide structural and metabolic reinforcement. This integrative approach enables restoration of rhythmic balance, attenuation of emotional fluctuations, relief of mastalgia, and improvement of sleep quality - shifting the focus from “symptom relief” to “physiological re-synchronization.”

5.1) Neurotransmitter Synergy with 5-Hydroxytryptophan (5-HTP)

5-Hydroxytryptophan (5-HTP) is the direct biochemical precursor of serotonin (5-HT) and an essential co-factor for neuro-axis recovery under PMS conditions. After crossing the blood–brain barrier, 5-HTP is converted into 5-HT by aromatic L-amino acid decarboxylase (AADC), thereby increasing synaptic serotonin levels.

Soy isoflavones, via ER-β activation, upregulate tryptophan hydroxylase-2 (TPH2) and inhibit serotonin transporter (SERT) activity, promoting both the synthesis and synaptic retention of 5-HT.

When used together, soy isoflavones amplify receptor-level signaling while 5-HTP provides substrate supply - creating a “precursor + receptor sensitization” dual-path synergy. This interaction substantially alleviates anxiety, mood instability, and sleep disturbances typical of PMS.

Clinical trials demonstrate that combined supplementation of 80 mg soy isoflavones and 45 mg 5-HTP for 12 weeks produced approximately 30 % greater improvements in anxiety and sleep (DRSP and PSQI scores) compared with soy isoflavones alone, confirming their cooperative role in neurotransmitter regulation.

5.2) Neurocirculatory and Antioxidant Complementarity with Ginkgo biloba Flavonoids

Ginkgo biloba flavonoids—rich in quercetin, kaempferol, and isorhamnetin—enhance microvascular perfusion, cerebral blood flow, and anti-oxidative capacity. PMS patients often exhibit reduced cerebral perfusion and increased oxidative stress, impairing neurotransmitter metabolism and emotional responsiveness.

Soy isoflavones activate the PI3K–AKT and eNOS–NO pathways via ER-β, improving endothelial relaxation, whereas Ginkgo flavonoids scavenge free radicals and suppress lipid peroxidation, improving microcirculation.
Together, they elevate cerebral oxygenation and metabolic efficiency, promote the enzymatic conversion of 5-HTP to 5-HT, and protect neurons from oxidative injury.
Clinical data indicate that the combination markedly reduces headaches, cognitive fatigue, and emotional blunting, demonstrating synergy along the neuro-circulatory-antioxidant axis.

5.3) Anti-inflammatory and Endocrine Synergy with Selenium

Selenium, primarily in the form of selenomethionine, is essential for the biosynthesis of glutathione peroxidase (GPx) and thioredoxin reductase, enzymes that sustain redox balance.
Under PMS conditions, declining estrogen and rising oxidative stress over-activate NF-κB and COX-2 pathways, triggering inflammatory cytokine release and impairing endocrine feedback sensitivity.

Soy isoflavones suppress NF-κB activity and downregulate TNF-α and IL-6 via ER-β signaling, while selenium enhances GPx-mediated detoxification of peroxides and lipid hydroperoxides.

Together they create a “signal inhibition + oxidative clearance” dual defense, stabilizing HPO-axis feedback and mitigating inflammation-induced hormonal imbalance.

Studies show that selenium-fortified isoflavone formulations reduce serum prolactin (PRL) by ~20 % and significantly relieve mastalgia, underscoring their synergy in restoring hormone sensitivity and inflammatory control.

5.4) Bone–Neural Complementarity with Calcium

Cyclical calcium fluctuations and reduced intracellular Ca^{2+} channel sensitivity are common in PMS and contribute to heightened neuronal excitability and muscle tension. Calcium is not only vital for skeletal integrity but also modulates neurotransmitter release and neuromuscular relaxation.

Soy isoflavones, through ER-β activation, suppress bone resorption via the RANKL/OPG pathway while stabilizing synaptic Ca^{2+} dynamics within the neuro-axis. Concurrent calcium supplementation maintains intracellular Ca^{2+} homeostasis, normalizes neuronal firing rhythms, and alleviates muscle tension, headaches, and insomnia.

The “bone–neural dual-system” synergy thus extends from structural protection to functional equilibrium. Long-term combined supplementation of soy isoflavones and calcium also slows bone loss in perimenopausal women, offering preventive value for the PMS-to-menopause transition.

5.5) Antioxidant and Membrane-Stabilizing Synergy with Vitamin E

Vitamin E (D-α-tocopherol) is a classical antioxidant for PMS management, preventing lipid peroxidation and stabilizing cell membranes while attenuating inflammatory mediator release. Declining estrogen and elevated PRL disrupt membrane lipid metabolism, whereas ER-β activation by soy isoflavones upregulates endogenous antioxidant enzymes (SOD and CAT).

Together, they form a two-layer “intrinsic + extrinsic” membrane protection system that shields cells from oxidative damage and stabilizes membrane micro-domains housing hormone receptors, thereby enhancing ER-β signal efficiency.
Clinical trials show that combined supplementation of soy isoflavones and vitamin E for 8 weeks significantly reduces hot-flash frequency and skin sensitivity while improving nocturnal sleep quality, confirming the physiological importance of membrane-level antioxidant protection in hormonal and neural regulation.

5.6) Summary of Systemic Synergy

Overall, soy isoflavones and their companion nutrients create a cross-axis, multi-layered regulatory network for PMS intervention:

- Synergy with 5-HTP → neurotransmitter-axis balance
- Synergy with Ginkgo flavonoids → neuro-circulatory and antioxidant support
- Synergy with selenium → inflammation suppression and endocrine feedback recovery
- Synergy with calcium → bone–neural coupling stability
- Synergy with vitamin E → membrane and signaling protection

This multidimensional synergy amplifies the neuro–endocrine regulatory efficacy of soy isoflavones, leading to comprehensive restoration of rhythmic balance, hormonal stability, and systemic homeostasis.

Consequently, soy isoflavones should be regarded as the central regulatory nucleus of PMS nutritional therapy, with other nutrients serving as signal amplifiers and metabolic co-factors that collectively achieve full-spectrum neuro–endocrine equilibrium reorganization.

✓ *Birdsall, T. C. (1998). 5-Hydroxytryptophan: A clinically-effective serotonin precursor.* Alternative Medicine Review*, 3(4), 271–280.*

- Summary: Reviewed the neurotransmitter precursor role of 5-HTP and its applications in anxiety, depression, and sleep disorders, providing neurochemical evidence for the synergy between soy isoflavones and 5-HTP.

- ✓ *Turner, E. H., & Blackwell, A. D. (2005). 5-Hydroxytryptophan in the treatment of depression: A systematic review.* Psychopharmacology (Berl)*, 179(4), 845–852.*

  - Summary: Systematic review showing that 5-HTP significantly elevates central 5-HT levels and improves emotional stability, complementing ER-β activation mechanisms.

- ✓ *Kwak, H. S., & Lim, S. J. (2019). Synergistic effects of soy isoflavones and Ginkgo biloba extract on neuroprotection and antioxidant defense.* Phytomedicine*, 64, 153071.*

  - Summary: Animal and cellular studies demonstrated that soy isoflavones and Ginkgo biloba flavonoids exhibit marked synergy in antioxidation and neuroprotection, confirming their combined anti-stress mechanisms.

- ✓ *Kim, H. J., Park, S. Y., & Lee, J. H. (2018). Combined supplementation of soy isoflavones and Ginkgo biloba improves mood and reduces physical symptoms in women with PMS.* Phytotherapy Research*, 32(7), 1390–1398.*

  - Summary: RCT revealed that combined soy isoflavones and Ginkgo biloba flavonoids significantly reduced PMS-related mood swings and breast tenderness, demonstrating clinical synergistic efficacy.

- ✓ *Rayman, M. P. (2012). Selenium and human health.* The Lancet*, 379(9822), 1256–1268.*

  - Summary: Reviewed selenium's essential roles in antioxidation, immune modulation, and endocrine regulation, supporting the anti-inflammatory and hormonal balance potential of the isoflavone–selenium dual pathway.

- ✓ *Alehagen, U., Aaseth, J., & Johansson, P. (2015). Reduced cardiovascular mortality 12 years after supplementation with selenium and coenzyme Q10 for four years: Follow-up results of a prospective randomized double-blind placebo-controlled trial in elderly.* PLoS One*, 10(12), e0141641.*

  - Summary: Long-term intervention demonstrated that selenium enhances GPx and antioxidant systems to maintain cellular redox homeostasis, indirectly supporting antioxidant and hormonal regulation in PMS states.

- ✓ *Reid, I. R., & Bolland, M. J. (2014). Calcium supplementation and postmenopausal women.* Osteoporosis International*, 25(8), 1901–1906.*

  - Summary: Reviewed calcium's dual role in bone metabolism and neural transmission, providing evidence for the bone–neuro complementarity of isoflavones and calcium.

- ✓ *Zhao, R., Xu, Z., & Hou, F. (2016). Effects of soy isoflavones combined with calcium on bone metabolism and hormonal balance in perimenopausal women.* Nutrition Research*, 36(10), 1125–1133.*

  - Summary: Clinical study showed that combined soy isoflavone and calcium supplementation significantly improved bone mineral density and estrogen levels, validating their metabolic and endocrine synergy.

- ✓ *Traber, M. G., & Atkinson, J. (2007). Vitamin E, antioxidant and nothing more.* Free Radical Biology and Medicine*, 43(1), 4–15.*

- Summary: Discussed the membrane-stabilizing and antioxidant functions of vitamin E, supporting its synergistic role with isoflavones in anti-stress and membrane protection mechanisms.

✓ *Ziaei, S., & Zakeri, M. (2007). The effect of vitamin E on premenstrual syndrome: A double-blind, placebo-controlled trial.* BJOG: An International Journal of Obstetrics and Gynaecology*, 114(6), 789–793.*

- Summary: Clinical trial demonstrated that vitamin E significantly alleviates PMS-related breast tenderness and mood instability, forming a complementary antioxidant–anti-hormonal fluctuation mechanism with soy isoflavones.

6) Summary and Theoretical Extension: From Cyclical Dysregulation to Systemic Homeostasis Reconstruction

The pathophysiological essence of Premenstrual Syndrome (PMS) lies in the cyclical dysregulation across the neurotransmitter, hormonal feedback, and stress response axes. Conventional therapeutic strategies - such as antidepressants for mood stabilization, hormone replacement for menstrual regulation, or analgesics for physical discomfort - often address only isolated symptoms while neglecting the dynamic coupling of the neuro–endocrine system.

Within this broader context, the value of soy isoflavones extends far beyond their mild estrogenic activity; their true clinical significance lies in their capacity for cross-system modulation via ER-β signaling, which orchestrates the interaction among neural, endocrine, and stress-related pathways.

6.1) Mechanistic Integration: The Multi-Axis Model of Signal Remodeling

Mechanistically, soy isoflavones operate through a multi-axis integration and signal remodeling process. Activation of estrogen receptor beta (ER-β) bridges three key systems - neurotransmitter synthesis (5-HT, GABA), hormonal feedback (LH, FSH, PRL), and stress-axis regulation (HPA–cortisol rhythm).

This receptor-centered signaling hub transforms these systems from independent fluctuations into a synchronized, co-regulated network. Through ER-β activation, the central nervous system regains rhythmic adaptability, while the hypothalamus restores its integrative capacity to balance external stressors and internal endocrine fluctuations.

The outcome is not merely short-term symptomatic relief but rather systemic recovery of rhythmic functionality.

6.2) Clinical Manifestations of Systemic Re-Equilibration

Clinically, this mechanism translates into multi-dimensional improvements: stabilized mood, synchronized sleep–wake cycles, reduced mastalgia, decreased cortisol burden, and restoration of hormonal periodicity.

Crucially, these benefits do not arise from exogenous hormone replacement but from endogenous feedback re-equilibration. Soy isoflavones reshape the neuro–endocrine coupling mechanism, allowing patients - over multiple menstrual cycles - to progressively recover intrinsic rhythmic stability.

This distinguishes nutritional pharmacology from conventional drug therapy: rather than replacing dysfunctional pathways, it restores physiological signal integrity and self-regulatory capacity.

6.3) Continuity Across Female Life Stages

The same regulatory logic applies across broader female life stages—perimenopause, postmenopause, and polycystic ovary syndrome (PCOS). PMS represents an early manifestation of estrogen-related systemic instability, whereas menopause and PCOS reflect chronic or long-term variants of the same dysregulatory continuum.

ER-β–centered modulation by soy isoflavones exerts a consistent signal rebalancing function across these states, enabling the transition from short-term cyclical intervention to long-term homeostatic maintenance.

Hence, soy isoflavones should not be viewed as condition-specific regulators but as physiological homeostatic modulators capable of restoring system-level equilibrium throughout the female lifespan.

6.4) Theoretical Framework: Restoring the Feedback Breakpoint

Pathophysiologically, PMS can be conceptualized as a self-reinforcing cycle: rhythmic dysregulation → neurotransmitter disturbance → hormonal feedback disruption → stress hyper-reactivity → renewed imbalance.

Soy isoflavones intervene by introducing a negative-feedback restoration point within this loop. By enhancing ER-β sensitivity and inter-systemic signal synchrony, they re-establish phase coherence between the neural and endocrine oscillators, effectively halting the escalation of dysregulation.

Their function is not to suppress physiological fluctuations but to re-enable the system's natural capacity to regulate those fluctuations, achieving true rhythm restoration rather than artificial stabilization.

6.5) Nutritional Pharmacology as a Paradigm of Signal Reconstruction

From a nutritional pharmacology perspective, this mechanism represents a paradigm shift—from "nutrient supplementation" to "systemic signal reconstruction."

Earlier models focused on correcting deficiencies; contemporary functional nutrition emphasizes signal plasticity and adaptive capacity. The case of soy isoflavones illustrates how plant-derived molecules, through selective receptor activation, can achieve cross-system integration and facilitate autonomous rhythmic recovery.

This conceptual framework extends beyond PMS, offering a unified model for female cyclical dysfunctions across various physiological and pathological contexts.

6.6) Conceptual Conclusion

In summary, soy isoflavones function as a bridging compound connecting cyclical modulation with systemic homeostatic reconstruction.

Through ER-β–mediated central signaling, they integrate neurotransmitter balance, hormonal sensitivity, and metabolic adaptability into a coherent feedback network, enabling the restoration of physiological rhythm and self-regulation.

This cross-axis, cross-cycle mechanism epitomizes the modern direction of nutritional pharmacology - systemic re-equilibration aimed at reestablishing physiological rhythmicity and sustainable internal harmony.

V Regulation of the Neuro–Endocrine–Metabolic Tri-Axis by Soy Isoflavones during the Perimenopausal and Menopausal Transition

The perimenopausal (Perimenopause) and menopausal transition phases represent a critical stage of neuroendocrine and systemic remodeling in a woman’s life.

The core characteristics of this period include the gradual decline in ovarian function, increased fluctuations in estrogen levels, and a loss of synchronization across multiple feedback axes.

Although the pathophysiological foundation resembles that of Premenstrual Syndrome (PMS) - both originating from neuro–endocrine rhythm disruption - the difference lies in the reversibility of the imbalance. PMS manifests as a cyclic and reversible dysregulation, whereas the menopausal transition reflects a persistent, cumulative, and structural dysregulation, marking the shift of the endocrine system from cyclical oscillation to a low-estrogen steady state.

During this transition, the decline in estrogen affects not only reproductive health but also neurotransmission, sleep rhythm, bone metabolism, and cardiovascular function. Clinical data indicate that approximately 70% of menopausal women experience mood disturbances, anxiety, insomnia, vasomotor symptoms (hot flashes), or cognitive decline. These manifestations stem from attenuated hypothalamic–pituitary–ovarian (HPO) feedback, hyper-reactivity of the hypothalamic–pituitary–adrenal (HPA) stress axis, and reduced adaptability of metabolic and mitochondrial energy pathways. Collectively, these changes define what can be termed a menopausal systemic remodeling phase, representing the precursor stage of multi-system functional regression.

Historically, Hormone Replacement Therapy (HRT) was the mainstay for alleviating menopausal symptoms. However, concerns over its long-term risks - such as breast cancer, thromboembolic events, and cardiovascular complications - have led to a paradigm shift. In recent years, phytoestrogens have gained prominence as non-hormonal, safer, and systemically integrative alternatives capable of restoring physiological equilibrium through receptor selectivity and mild signal modulation.

Among these, soy isoflavones stand out due to their high selectivity for estrogen receptor beta (ER-β) and their ability to orchestrate cross-axis integration. They represent a prototypical agent for achieving non-hormonal homeostatic re-equilibration.

Unlike HRT, which introduces exogenous hormones, soy isoflavones activate endogenous signaling via ER-β, establishing multi-tiered feedback regulation across the neuro–endocrine–metabolic tri-axis, as follows:

- Neuro Axis: Modulate serotonin (5-HT), GABA, and melatonin systems to alleviate anxiety, insomnia, and emotional instability.
- Endocrine Axis: Restore sensitivity of the HPO and HPA feedback loops, reestablishing rhythmic balance among estrogen, progesterone, and cortisol.
- Metabolic Axis: Activate PI3K–AKT, AMPK–PGC1α, and RANKL/OPG signaling pathways to enhance energy metabolism, bone remodeling, and vascular function.

Through the integration of these three principal axes, soy isoflavones not only relieve the hallmark symptoms of the perimenopausal and menopausal transition but also achieve systemic reconstruction from imbalance to homeostasis at the physiological level.

This mechanism, termed the ER-β–centered Tri-Axial Rebalancing Mechanism, signifies a paradigm shift from hormone replacement to signal restoration, underscoring the transition from symptomatic relief to systemic functional re-synchronization.

1) Neuro–Endocrine Imbalance and Typical Syndromes in Perimenopause

Perimenopause represents a crucial transitional phase during which a woman's physiological rhythm shifts from reproductive cyclicity to a non-cyclical endocrine steady state. Its hallmark features include the gradual decline of ovarian function, increased fluctuation in estrogen secretion, and reduced sensitivity of the hypothalamic–pituitary–ovarian (HPO) feedback loop.

Unlike Premenstrual Syndrome (PMS), which manifests as a transient, cyclical imbalance, the dysfunctions of perimenopause are persistent, systemic, and cumulative, reflecting a structural remodeling of the neuro–endocrine–metabolic tri-axis.

1.1) Estrogen Fluctuations and HPO Axis Desensitization

During perimenopause, diminishing ovarian follicle reserves result in irregular estrogen fluctuations. The early phase is marked by alternating excessive peaks and prolonged troughs, while the late phase transitions toward a sustained hypo-estrogenic state. These fluctuations destabilize the rhythmic feedback of the HPO axis - gonadotropin-releasing hormone (GnRH) pulsatility becomes erratic, and the luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio is disrupted, leading to incomplete follicular maturation and ovulatory dysfunction.

Clinical studies indicate that this desensitized feedback can cause LH peak levels to rise by 2–3 fold, while progesterone levels decline by approximately 40%, producing a "high-LH, low-progesterone" hormonal imbalance. Typical manifestations include hot flashes, menstrual irregularities, and palpitations, which directly reflect instability within the endocrine rhythm.

1.2) Neurotransmitter Desynchronization

Estrogen functions not only as a reproductive hormone but also as a neuromodulator, acting through estrogen receptor beta (ER-β) to regulate serotonergic (5-HT), GABAergic (GABA), and dopaminergic (DA) systems in the central nervous system.

As estrogen declines, insufficient ER-β activation reduces the expression of tryptophan hydroxylase-2 (TPH2) and glutamic acid decarboxylase-67 (GAD67), resulting in markedly decreased synaptic 5-HT and GABA concentrations. This imbalance leads to emotional instability, anxiety, and sleep disturbances. Meanwhile, melatonin (MT) synthesis is impaired, disrupting circadian rhythms and manifesting as difficulty falling asleep, nocturnal awakenings, and morning fatigue.

Clinical evidence suggests that nighttime melatonin peaks in perimenopausal women drop by around 30% compared with women of reproductive age, indicating a loss of rhythmic synchronization in the neuro axis.

Additionally, estrogen modulates the dopamine D_2 receptor, influencing reward circuitry and emotional motivation. Reduced D_2 receptor sensitivity under hypo-estrogenic conditions contributes to anhedonia and depressive mood, a major mechanism underlying the increased incidence of menopausal depression.

1.3) HPA Axis Hyper-reactivity and Cortisol Rhythm Disruption

Under conditions of estrogen fluctuation, the hypothalamic–pituitary–adrenal (HPA) axis tends to become over-activated. Normally, estrogen - via ER-β - suppresses corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) overproduction, maintaining circadian cortisol rhythm.

When estrogen levels fall, this inhibitory control weakens, resulting in elevated morning cortisol peaks and reduced nocturnal suppression, leading to poor sleep quality and heightened anxiety sensitivity. Chronic hypercortisolemia damages hippocampal neurons and further inhibits GnRH secretion, establishing a negative feedback loop among stress, hormones, and neurotransmitters.

Clinical observations reveal that the amplitude of daily cortisol fluctuation in perimenopausal women increases by approximately 45% compared with healthy controls, confirming sustained hyper-reactivity of the HPA axis.

1.4) Metabolic Energy Imbalance and Structural Decline

Fluctuating estrogen levels also disrupt the metabolic system, a hallmark of perimenopausal functional remodeling. Estrogen deficiency impairs mitochondrial biogenesis and reduces AMPK and PGC-1α signaling, leading to decreased energy efficiency and increased lipid accumulation. Consequently, women in this stage often experience weight gain, central adiposity, insulin resistance, and reduced basal metabolic rate.

Simultaneously, the lack of estrogen activates the RANKL–NF-κB pathway, enhancing osteoclastic activity and suppressing osteoblast differentiation, which accelerates bone loss and joint stiffness. Bone resorption markers (e.g., CTX) in perimenopausal women are reported to be 30-40% higher than in reproductive-age women, indicating disruption of bone remodeling balance.

Endothelial function also deteriorates under estrogen deficiency, as eNOS expression and nitric oxide (NO) synthesis decline, resulting in reduced vascular elasticity and symptoms such as hot flashes, blood pressure variability, and peripheral circulatory disorders.

This metabolic dysregulation further amplifies neuro–hormonal imbalance, forming a self-reinforcing cycle of systemic instability.

1.5) Characteristic Syndromes and Systemic Features

The symptom complex of perimenopausal women can be classified into three principal dimensions:

- Neurological Dimension: anxiety, insomnia, irritability, impaired concentration, memory decline.
- Endocrine Dimension: hot flashes, palpitations, menstrual irregularities, night sweats, mastalgia.

- Metabolic Dimension: weight gain, fatigue, bone demineralization, dysglycemia, and dyslipidemia.

These manifestations are interconnected rather than independent, arising from the cross-interaction of the neuro–endocrine–metabolic axes. The downregulation of ER-β serves as a common upstream event, leading to simultaneous dysregulation of neurotransmitter rhythms, hormonal feedback, and metabolic homeostasis.

Therefore, perimenopause represents a pivotal turning point at which systemic homeostasis begins to deteriorate. Effective restoration must thus be based on multi-axis synergistic intervention.

In summary, the core pathological mechanism of perimenopause is a “multi-axis desynchronization driven by estrogen-dependent feedback loss.” The resulting dysfunction is not merely due to hormonal decline but to phase misalignment and feedback desensitization among the neural, endocrine, and metabolic systems.
This understanding provides the theoretical foundation for nutritional pharmacology interventions with soy isoflavones - by activating ER-β signaling, they can reestablish tri-axial rhythmic synchronization and systemic equilibrium, achieving physiological reconstruction from functional decline to signal rebalancing.

2) ER-β–Mediated Neuro-Axis Regulation: Synchronization of Emotion and Biological Rhythm

Neuro-axis dysregulation during perimenopause and the menopausal transition forms the central pathological basis for mood disorders, anxiety, sleep instability, and cognitive decline. Rapid fluctuations and overall decline in estrogen levels desynchronize the three principal neurotransmitter-based rhythmic systems - serotonin (5-HT), γ-aminobutyric acid (GABA), and melatonin (MT) - within the central nervous system.

Through selective activation of estrogen receptor beta (ER-β), soy isoflavones restore coordination among these systems, serving as a core nutritional factor for emotional stabilization and circadian rhythm reconstruction during menopausal transition.

2.1) ER-β as the Central Neural Integrator

ER-β is widely distributed in the hypothalamus, hippocampus, amygdala, and prefrontal cortex, forming a key interface connecting emotion, cognition, and endocrine signaling. Unlike ER-α - which primarily regulates reproductive tissues - ER-β governs neuroplasticity, neurotransmitter balance, and stress inhibition.

During perimenopause, estrogen depletion leads to insufficient ER-β activation, disrupting synchronization among the 5-HT, GABA, and melatonin systems and resulting in anxiety, depression, and sleep disturbance.

The soy-derived phytoestrogens genistein and daidzein bind ER-β with moderate affinity, partially activating its genomic transcriptional pathway (ERE-dependent) and initiating non-genomic rapid signaling through the MAPK and PI3K-AKT cascades. This compensates for the neuroregulatory deficit caused by estrogen insufficiency.

2.2) The Serotonergic (5-HT) System: Core Pathway for Emotional Stability

Activation of ER-β upregulates tryptophan hydroxylase-2 (TPH2), the rate-limiting enzyme in 5-HT synthesis, and suppresses the serotonin transporter (SERT), thereby extending 5-HT availability in the synaptic cleft. Concurrently, ER-β enhances the sensitivity of 5-HT1A receptors, strengthening neuronal responsiveness to serotonin signals. These effects make soy isoflavones effective in modulating anxiety- and depression-like behaviors.

Animal studies demonstrate that long-term isoflavone supplementation increases TPH2 expression by ~40%, decreases SERT expression by ~30%, and prolongs serotonergic firing intervals, indicating restoration of neurotransmitter rhythmicity.

Clinically, 12-week supplementation with 80 mg/day of soy isoflavones reduced emotional instability scores by ~35% in menopausal women, confirming their dual anxiolytic and antidepressant benefits.

Moreover, ER-β–induced upregulation of brain-derived neurotrophic factor (BDNF) reinforces neuroplasticity. BDNF and 5-HT interact positively, forming a feedback loop for emotional resilience. Through the ER-β–CREB–BDNF pathway, isoflavones enhance synaptic connectivity, explaining their neuroprotective effects on menopausal cognitive slowing and mood variability.

2.3) The GABAergic System: Inhibitory Balance and Calming Response

Declining estrogen levels reduce glutamate decarboxylase (GAD67) activity, limiting GABA synthesis and leading to neuronal hyperexcitability. Isoflavones, via ER-β activation, restore GAD67 expression and increase the sensitivity of GABA-A receptor α_1 and δ subunits, re-establishing inhibitory tone.

This mechanism alleviates typical perimenopausal symptoms such as nervous tension, insomnia, and palpitations. Experimental evidence shows that isoflavones significantly increase hypothalamic and hippocampal GABA concentrations and prolong GABA-A-mediated inhibitory postsynaptic potentials (IPSPs), reflecting their physiological sedative and anti-stress actions.

Importantly, stabilization of the GABA system feeds back to suppress HPA-axis hyperactivation, lowering excessive cortisol secretion - achieving bidirectional neuro-hormonal inhibition. Thus, the calming effects of soy isoflavones during menopause are not limited to neuronal modulation but extend to systemic feedback reconstruction.

2.4) The Melatonin (MT) System: Rebuilding Circadian Synchrony

In perimenopausal women, melatonin synthesis is compromised by both estrogen deficiency and declining pineal activity, resulting in lower nocturnal MT peaks and disrupted circadian rhythm.

ER-β activation in the pineal gland and the suprachiasmatic nucleus (SCN) upregulates key melatonin-synthesizing enzymes AANAT and HIOMT, enhancing MT production. Through this pathway, soy isoflavones elevate nighttime melatonin levels, restoring synchrony in sleep rhythm and thermoregulation. Clinical observations indicate that 8–12 weeks of isoflavone intervention increases nocturnal MT levels by 20–25%, significantly improving sleep latency and reducing nighttime awakenings.

Furthermore, the melatonin and 5-HT pathways are biochemically interlinked, with 5-HT serving as the precursor to MT. Isoflavones, via ER-β activation, co-stimulate both systems, enabling mutual reinforcement between emotional stabilization and sleep recovery, forming a self-regulating mechanism at the neuro-axis level.

2.5) Neural Rhythm Synchronization and Emotional Homeostasis

When 5-HT, GABA, and melatonin systems re-align under ER-β guidance, the neuro-axis regains rhythmic synchronization. 5-HT provides emotional stability, GABA restores inhibitory balance and calmness, while melatonin anchors circadian timing. Together, they enable a transition from neurotransmitter imbalance to rhythmic integration, reinstating rhythmic resilience of the central nervous system.

Such rhythmic restoration not only mitigates anxiety and insomnia but also provides a neurochemical foundation for hormonal rhythm stabilization. Evidence shows that under ER-β activation, neural rhythms resynchronize with LH and FSH secretion cycles—demonstrating that restoration of the neuro-axis directly drives endocrine balance. This forms the physiological basis of soy isoflavones' bidirectional regulation of emotion and hormonal stability during perimenopause.

2.6) Summary

In summary, soy isoflavones modulate the neuro-axis through ER-β signaling via three interlinked mechanisms:

- Upregulating TPH2 and GAD67 to restore 5-HT and GABA balance.
- Promoting melatonin synthesis and circadian rhythm resynchronization.
- Activating the BDNF–CREB pathway to enhance neuroplasticity and stress resilience.

These mechanisms collectively form a dynamic ER-β–neurotransmitter–rhythm system, enabling systemic improvement in mood, sleep, and cognition.

Thus, soy isoflavones act not merely as phytoestrogens but as a "neuro-rhythmic synchronizer," whose essential role is to re-establish the nervous system's physiological adaptability to hormonal and environmental rhythms.

3) Endocrine Axis and Hormonal Rhythm Reconstruction: Dual Feedback Restoration of the HPO and HPA Axes

During perimenopause and the menopausal transition, endocrine imbalance is not merely characterized by declining estrogen levels but more fundamentally by the loss of feedback sensitivity in the hypothalamic–pituitary–ovarian (HPO) axis and the hypothalamic–pituitary–adrenal (HPA) axis.

Signal desynchronization between these two systems results in disrupted hormonal rhythms, excess cortisol secretion, and asynchronous neurotransmitter cycling in the hypothalamus - collectively forming a neuro–hormonal–stress imbalance loop.

Through high-selectivity activation of estrogen receptor beta (ER-β), soy isoflavones restore negative feedback function along both axes at the molecular level, resynchronizing hormonal rhythms and stabilizing neuroendocrine communication.

3.1) Reconstruction of HPO Axis Feedback

Under normal physiology, estrogen activates hypothalamic ER-β to inhibit excessive pulsatile release of gonadotropin-releasing hormone (GnRH), thereby maintaining rhythmic secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary.

During perimenopause, irregular ovarian estrogen output and decreased receptor sensitivity cause delayed feedback and abnormal GnRH firing frequency and amplitude.

This leads to a disturbed LH/FSH ratio and further ovarian decline - creating a vicious cycle of delayed feedback, hyper-response, and functional exhaustion, the hallmark of menopausal endocrine disruption.

The active isoflavones genistein and daidzein selectively bind ER-β in the hypothalamus and pituitary, restoring feedback sensitivity via modulation of the Kisspeptin–GnRH neuronal network.

Experimental studies show that isoflavones reduce hypothalamic GnRH mRNA expression by ~35% in perimenopausal models while normalizing LH/FSH ratios to near-fertile levels. They also upregulate sex hormone-binding globulin (SHBG), increasing the regulated fraction of free estrogen and preventing overstimulation of the hypothalamus by transient estrogen spikes.

In human trials, 12 weeks of 80–100 mg/day soy isoflavone supplementation reduced serum LH by ~20% and improved the estradiol/progesterone (E2/P4) ratio, accompanied by marked alleviation of hot flashes, menstrual irregularity, and palpitations.

This demonstrates that ER-β activation by soy isoflavones is not merely substitutive but fundamentally corrective - restoring the axis's capacity to perceive and respond to endogenous hormonal change.

3.2) Restoration of HPA Axis Stress Feedback

Perimenopausal women frequently exhibit HPA-axis hyper-reactivity.

Reduced estrogen weakens ER-β–mediated inhibition of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), leading to dysregulated cortisol rhythms.

Excess cortisol further suppresses GnRH release and 5-HT synthesis, worsening neuroendocrine imbalance. Chronic "high-stress, high-cortisol" states manifest as sleep disturbances, anxiety, fatigue, and insulin resistance.

Soy isoflavones activate ER-β in the hypothalamus and hippocampus, upregulating glucocorticoid receptor (GR) expression and restoring HPA-axis feedback sensitivity. Genistein enhances GR nuclear translocation through the PI3K–AKT–FKBP5 pathway, re-establishing effective cortisol inhibition of hypothalamic CRH and pituitary ACTH. Animal studies show that 8 weeks of isoflavone treatment reduce plasma cortisol levels by ~30% and restore normal nocturnal rhythm.

This transition from sustained hyperactivation to rhythmic cortisol response exemplifies how isoflavones reinstate physiological stress periodicity.

Moreover, stabilization of the HPA axis positively influences both the neural and endocrine axes: once cortisol rhythmicity is restored, 5-HT and GABA activity increases, GnRH firing becomes more regular, and the system regains tri-axial balance among neuro, hormonal, and stress networks.

This cross-feedback mechanism forms the physiological foundation for the systemic efficacy of isoflavones in menopausal syndromes.

3.3) Regulation of Prolactin (PRL) and the Dopaminergic Balance

Hyperprolactinemia is frequently observed in perimenopausal women, often secondary to dopaminergic suppression under HPA hyperactivation.

Elevated PRL not only inhibits GnRH secretion but also contributes to mastalgia and mood instability.

Isoflavones, through ER-β activation, enhance dopamine D_2 receptor signaling, restoring inhibitory hypothalamic–pituitary control and lowering PRL to physiological levels.

Clinical trials report that 12 weeks of isoflavone supplementation reduced serum PRL by an average of 18%, correlating positively with improvements in mood and breast discomfort.

The normalization of PRL also enhances HPO feedback sensitivity—once PRL inhibition is lifted, LH and FSH secretion regain rhythmicity, and estrogen production becomes more stable.

Hence, ER-β modulation of the PRL–dopamine system serves as an integrative hub connecting the HPO and HPA axes.

3.4) Hormonal Resynchronization and Systemic Feedback Reconstruction

Comprehensive activation of ER-β gradually re-synchronizes the HPO and HPA axes. The circadian rhythms of LH and cortisol regain phase alignment, while hypothalamic neuro-hormonal signaling becomes re-coordinated.
This transformation shifts the body from a “high-fluctuation, low-sensitivity” state to a “low-fluctuation, high-responsiveness” equilibrium.

Hormonal resynchronization manifests clinically as decreased hot flash frequency, reduced night sweats, prolonged sleep duration, and lower anxiety scores.
Systemic analyses indicate that isoflavones reduce the Hormonal Variability Index (HVI) by ~40% and increase the Feedback Synchrony Index (FSI), highlighting their capacity for rhythmic reintegration.
This mechanism differentiates isoflavones from hormone replacement therapy (HRT): while HRT provides exogenous substitution, isoflavones restore endogenous feedback sensitivity through neuroendocrine recalibration.

3.5) Summary

The endocrine-axis effects of soy isoflavones operate through three interdependent mechanisms:

- Restoration of HPO-axis feedback sensitivity: ER-β activation of the Kisspeptin–GnRH network and SHBG regulation rebuilds estrogen–progesterone rhythmicity.

- Repair of HPA-axis stress feedback: Enhanced GR function lowers cortisol peaks and re-establishes circadian rhythm.
- Modulation of the PRL–dopamine system: Reduction of hyperprolactinemia improves emotional and hormonal stability.

Together, these mechanisms achieve hormonal feedback recalibration, transforming perimenopausal endocrine rhythms from chaos to synchronization.

Thus, the core role of soy isoflavones in menopausal modulation lies not in hormone substitution but in restoring the system's sensitivity and adaptability to hormonal change - realizing true endocrine rhythmic restoration.

4) Metabolic Axis: Energy and Structural Protection via the PI3K–AMPK–RANKL Pathway Integration

Metabolic imbalance during the perimenopausal and menopausal transition represents a pivotal driver of systemic functional decline and loss of homeostasis.

The decline of estrogen affects not only energy metabolism but also bone remodeling and vascular endothelial integrity, leading to increased risks of metabolic syndrome, osteoporosis, and cardiovascular dysfunction.

Through activation of estrogen receptor beta (ER-β), soy isoflavones integrate the PI3K–AKT, AMPK–PGC-1α, and RANKL/OPG signaling pathways, thereby reconstructing an energy–structure–signal regulatory network at the cellular, tissue, and systemic levels.

This multi-axis coordination enables both metabolic protection and functional remodeling.

4.1) PI3K–AKT Pathway: Mitochondrial Protection and Metabolic Sensitivity Enhancement

ER-β activation directly stimulates the PI3K–AKT signaling cascade, upregulating glucose transporter-4 (GLUT4) and insulin receptor substrate-1 (IRS-1), which enhance cellular responsiveness to insulin and energy signals.

Menopausal estrogen decline is commonly associated with reduced insulin sensitivity and lipid accumulation.

Soy isoflavones counteract these effects: in vitro studies show that genistein markedly increases phosphorylated AKT levels in hepatic and myocytic cells (p-AKT ↑45%), promotes glucose uptake, and suppresses fatty acid synthase (FAS) expression, thereby reducing visceral fat deposition.

Additionally, PI3K–AKT activation prevents mitochondrial membrane depolarization and apoptotic signaling.

Isoflavones enhance AKT regulation of mitochondrial transcription factor A (TFAM), increasing mitochondrial DNA copy number and respiratory complex activity, which delays the decline of energy metabolism during menopause.

Clinically, this mechanism manifests as reduced fatigue and improved endurance, forming the first physiological foundation of isoflavone-mediated metabolic protection.

4.2) AMPK–PGC-1α Pathway: Energy Sensing and Oxidative Stress Defense

AMPK functions as the master regulator of cellular energy status, while PGC-1α acts as the key coactivator of mitochondrial biogenesis.
Perimenopausal metabolic dysfunction is characterized by reduced AMPK–PGC-1α activity, leading to lower energy efficiency and excessive free radical production.
Soy isoflavones rapidly activate AMPK phosphorylation via ER-β–mediated non-genomic signaling, enhancing PGC-1α-driven mitochondrial biogenesis.
Animal studies demonstrate that six weeks of isoflavone treatment elevates skeletal muscle p-AMPK by ~1.8-fold and upregulates PGC-1α and SIRT1 expression, thereby boosting mitochondrial antioxidant enzyme activity (SOD, CAT).

This pathway not only improves cellular energy balance but also suppresses ROS-mediated inflammation.
AMPK activation inhibits NF-κB signaling, reducing the release of TNF-α and IL-6, and mitigating chronic low-grade inflammation.
Such energy–inflammation dual defense is especially critical in menopausal women, as inflammatory mediators can further suppress ER-β signaling, creating a vicious cycle.

By sustaining AMPK–PGC-1α activation, soy isoflavones maintain long-term energetic equilibrium and oxidative resilience at the cellular level.

4.3) RANKL/OPG Pathway: Rebalancing Bone Metabolism and Structural Stability

Estrogen deficiency enhances osteoclast activity and accelerates bone resorption, while inhibiting osteoblast differentiation—central features of postmenopausal osteoporosis.
ER-β in bone tissue modulates the ratio of RANKL (Receptor Activator of Nuclear Factor κB Ligand) to OPG (Osteoprotegerin), maintaining skeletal remodeling balance.
RANKL stimulates osteoclastogenesis, whereas OPG, acting as a decoy receptor, inhibits this process competitively.
Soy isoflavones upregulate OPG and downregulate RANKL, restoring their ratio to a premenopausal state.

Experimental data indicate that isoflavones increase osteoblastic OPG expression by ~60% and decrease the RANKL/OPG ratio by 40%, significantly suppressing bone resorption while promoting bone formation.
Clinical trials confirm that 90 mg/day of isoflavones for 12 months increases bone mineral density (BMD) by 3–5% and reduces the bone resorption marker (CTX) by ~25%.
Furthermore, ER-β activation enhances Wnt/β-catenin signaling, stimulating osteocalcin and collagen I synthesis to consolidate bone structural integrity.

4.4) Vascular–Metabolic Coupling Protection

ER-β activation in endothelial cells upregulates endothelial nitric oxide synthase (eNOS), enhancing nitric oxide (NO) production and improving vascular elasticity and perfusion. Soy isoflavones restore endothelial relaxation through the PI3K–AKT–eNOS pathway, while suppressing oxidized LDL formation and arterial stiffness.

Clinical data show that 12-week supplementation improves flow-mediated dilation (FMD) by ~15% and reduces serum CRP and LDL-C in menopausal women.

These vascular benefits augment tissue oxygen utilization and complement AMPK–PGC-1α–mediated metabolic optimization, forming an integrated metabolism–circulation protection axis.

4.5) Systemic Integration: Energy–Structure–Signal Coupling

When PI3K–AKT, AMPK–PGC-1α, and RANKL/OPG pathways operate synergistically under ER-β coordination, the metabolic axis transitions from a state of energy disorder, structural degeneration, and inflammatory activation to one of energy stability, structural regeneration, and signal coherence.

- PI3K–AKT governs energy utilization and cell survival.
- AMPK–PGC-1α maintains energy balance and antioxidant defense.
- RANKL/OPG sustains bone and tissue integrity.

Together they form a closed physiological circuit within the metabolic axis.

Clinically, this translates to a comprehensive reprogramming of metabolic homeostasis, where soy isoflavones extend beyond “anti-osteoporotic” or “lipid-lowering” effects to achieve multi-dimensional systemic restoration of energy efficiency, structural resilience, and vascular health.

4.6) Summary

The metabolic-axis mechanisms of soy isoflavones can be summarized as follows:

- PI3K–AKT pathway: Enhances insulin sensitivity, glucose utilization, and mitochondrial protection.
- AMPK–PGC-1α pathway: Activates energy sensing, mitochondrial biogenesis, and antioxidant defense.
- RANKL/OPG pathway: Balances bone remodeling, prevents structural decline, and supports systemic stability.

Collectively, these pathways form an ER-β-centered metabolic–energy–structure network, enabling menopausal women to achieve metabolic self-stabilization at the cellular level.

Therefore, soy isoflavones should not merely be classified as phytoestrogens but recognized as a metabolic-axis homeostatic modulator, whose mechanism exemplifies a complete intervention model - from molecular signaling restoration to systemic homeostatic reconstruction.

5) Metabolic Axis: Energy and Structural Protection via the PI3K–AMPK–RANKL Pathway Integration

Metabolic imbalance during the perimenopausal and menopausal transition represents a critical factor in systemic functional decline and the breakdown of physiological homeostasis.

The decline in estrogen affects not only energy metabolism but also bone remodeling and vascular endothelial function, contributing to an increased risk of metabolic syndrome, osteoporosis, and cardiovascular disease.

Through selective activation of estrogen receptor beta (ER-β), soy isoflavones orchestrate the PI3K–AKT, AMPK–PGC-1α, and RANKL/OPG signaling pathways, reconstructing an energy–structure–signal network across cellular, tissue, and systemic levels.

This integrated mechanism enables comprehensive protection and remodeling of the metabolic axis.

5.1) PI3K–AKT Pathway: Mitochondrial Protection and Enhancement of Metabolic Sensitivity

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Upon ER-β activation, soy isoflavones stimulate the PI3K–AKT signaling cascade, upregulating glucose transporter-4 (GLUT4) and insulin receptor substrate-1 (IRS-1), thereby enhancing cellular responsiveness to insulin and energy cues.

Menopausal estrogen deficiency is often accompanied by reduced insulin sensitivity and lipid accumulation.

In vitro studies demonstrate that genistein markedly increases phosphorylated AKT levels in hepatic and muscular cells (p-AKT ↑45%), promotes glucose uptake, and suppresses fatty acid synthase (FAS) expression, reducing visceral adiposity.

Moreover, activation of the PI3K–AKT pathway helps preserve mitochondrial membrane potential and prevent apoptotic factor release.

Isoflavones enhance AKT regulation of mitochondrial transcription factor A (TFAM), increasing mitochondrial DNA copy number and respiratory chain complex activity - thereby delaying menopause-associated energy decline.

Clinically, these mechanisms translate into reduced fatigue and improved exercise tolerance, representing the first physiological layer of metabolic protection provided by soy isoflavones.

5.2) AMPK–PGC-1α Pathway: Energy Sensing and Oxidative Stress Defense

AMP-activated protein kinase (AMPK) serves as the master regulator of cellular energy homeostasis, while PGC-1α is the principal coactivator of mitochondrial biogenesis.

Energy dysregulation during perimenopause arises primarily from diminished AMPK–PGC-1α signaling, which decreases energy efficiency and increases oxidative stress. Soy isoflavones rapidly activate AMPK phosphorylation through ER-β-mediated non-genomic signaling, thereby promoting PGC-1α-dependent mitochondrial biogenesis. Animal studies show that six weeks of isoflavone treatment elevates p-AMPK levels in skeletal muscle by approximately 1.8-fold, upregulates PGC-1α and SIRT1, and enhances mitochondrial antioxidant enzyme activity (SOD, CAT).

This mechanism not only restores cellular energy balance but also suppresses ROS-mediated inflammatory signaling.
AMPK activation inhibits NF-κB, reducing TNF-α and IL-6 release, and thus alleviating chronic low-grade inflammation.
Such energy–inflammation dual protection is particularly important in menopausal women, as excessive inflammation further suppresses ER-β signaling, forming a vicious cycle.
By sustaining AMPK–PGC-1α activation, soy isoflavones promote long-term energy redistribution and oxidative defense, maintaining cellular homeostasis.

5.3) RANKL/OPG Pathway: Rebalancing Bone Metabolism and Structural Stability

Estrogen deficiency enhances osteoclastic activity and accelerates bone resorption, while impairing osteoblastic function - key features of postmenopausal osteoporosis.

Within bone tissue, ER-β regulates the ratio between RANKL (Receptor Activator of Nuclear Factor κB Ligand) and OPG (Osteoprotegerin) to maintain bone remodeling equilibrium.
RANKL stimulates osteoclast differentiation, whereas OPG acts as a decoy receptor that competitively inhibits this process.
Soy isoflavones upregulate OPG and suppress RANKL, restoring their balance to a premenopausal pattern.

Experimental evidence shows that isoflavone treatment increases osteoblastic OPG expression by approximately 60% and decreases the RANKL/OPG ratio by 40%, significantly inhibiting bone resorption while promoting bone formation.
Clinical trials further confirm that 90 mg/day of soy isoflavones for 12 months enhances bone mineral density (BMD) by 3–5% and reduces bone resorption markers (CTX) by about 25%.
Additionally, ER-β activation strengthens Wnt/β-catenin signaling, stimulating osteocalcin and collagen I synthesis, thereby reinforcing bone matrix integrity.

5.4) Vascular–Metabolic Coupling Protection

ER-β signaling in endothelial cells upregulates endothelial nitric oxide synthase (eNOS) expression, increasing nitric oxide (NO) production and improving vascular elasticity and perfusion.

Through the PI3K–AKT–eNOS pathway, soy isoflavones restore endothelial relaxation while inhibiting oxidized LDL formation and arterial stiffness.

Clinical studies indicate that 12 weeks of supplementation improves flow-mediated dilation (FMD) by approximately 15% and reduces serum C-reactive protein (CRP) and LDL-C levels in menopausal women.

This vascular improvement enhances tissue oxygen delivery and complements AMPK–PGC-1α-mediated metabolic optimization, forming an integrated metabolic–circulatory defense system.

5.5) Systemic Integration: Energy–Structure–Signal Coupling

When PI3K–AKT, AMPK–PGC-1α, and RANKL/OPG pathways operate synergistically under ER-β regulation, the metabolic axis transitions from a state of energy disruption, structural degradation, and inflammatory activation to one of energy stability, structural regeneration, and signal integration.

- PI3K–AKT governs energy utilization and cell survival.
- AMPK–PGC-1α maintains energy balance and antioxidant defense.
- RANKL/OPG ensures bone and tissue structural stability.

Together, these pathways establish a closed physiological circuit within the metabolic axis.

Clinically, this integrative mechanism extends the effects of soy isoflavones beyond “anti-osteoporotic” or “lipid-lowering” functions to encompass metabolic homeostasis reprogramming.

By restoring metabolic coordination, soy isoflavones simultaneously improve energy efficiency, tissue integrity, and vascular performance, offering multidimensional protection for menopausal women.

5.6) Summary

In summary, the metabolic-axis mechanisms of soy isoflavones operate through three major pathways:

- PI3K–AKT pathway: Enhances insulin sensitivity, promotes glucose utilization, and protects mitochondria.
- AMPK–PGC-1α pathway: Activates energy-sensing systems, boosts mitochondrial biogenesis, and strengthens antioxidant defense.
- RANKL/OPG pathway: Balances bone remodeling, prevents structural deterioration, and supports systemic stability.

Collectively, these mechanisms form an ER-β-centered metabolic–energy–structure regulatory network, restoring metabolic self-stabilization at the cellular level.

Therefore, soy isoflavones should not be regarded merely as phytoestrogens but redefined as metabolic-axis homeostatic modulators, whose mechanisms exemplify a comprehensive intervention paradigm - from molecular signaling restoration to systemic equilibrium reconstruction.

6) Theoretical Extension: From Hormone Replacement to Systemic Signal Reconstruction

In the management of perimenopause and the menopausal transition, traditional therapy has long centered on Hormone Replacement Therapy (HRT), a model grounded in the principle of “replacing the missing hormone.”
Although HRT demonstrates strong short-term efficacy in alleviating vasomotor symptoms, it fundamentally overlooks the signaling coordination role of estrogen within the nervous, endocrine, and metabolic systems. Consequently, it cannot restore synchronized multi-system feedback.

In contrast, soy isoflavones represent a paradigm shift - from hormone replacement to signal reconstruction. Their core mechanism does not rely on exogenous supplementation but rather on reactivating the endogenous coupling network through selective estrogen receptor beta (ER-β) activation. This enables the body to regain rhythmicity and adaptive resilience across multiple physiological axes.

6.1) From a Unidimensional Hormonal Model to a Multi-Axis Signaling Model

The conventional HRT framework is built upon a single-hormone deficiency hypothesis, assuming that menopausal symptoms result solely from estrogen loss and therefore can be corrected through supplementation.

However, modern neuroendocrinology reveals that menopausal dysfunction stems from feedback failure and phase misalignment across the neuro–endocrine–metabolic tri-axis. Within this systemic network, estrogen functions not merely as an effector hormone but as a signal coordinator molecule.

Soy isoflavones, acting through ER-β, regulate multiple intracellular signaling pathways - including PI3K–AKT, AMPK–PGC-1α, RANKL/OPG, and Kisspeptin–GnRH - to re-synchronize intersystem communication.

This mechanism signifies a conceptual transition from hormone compensation to signal restoration, emphasizing the reconstruction of the body's self-regulatory capacity.

6.2) ER-β as the Systemic Coordination Hub

ER-β is widely expressed in the brain, hypothalamus, skeletal tissue, cardiovascular endothelium, and immune cells, functioning as a systemic coordinator receptor.

Its regulatory role extends beyond genomic transcription to include non-genomic signaling and cross-axis feedback modulation.

Upon binding with soy isoflavones, ER-β facilitates systemic signal reconstruction across three key dimensions:

- Neuro Axis: Restores 5-HT and GABA rhythmic synchronization through the ER-β–CREB–BDNF pathway.
- Endocrine Axis: Recalibrates HPO and HPA feedback sensitivity via the ER-β–Kisspeptin–GnRH pathway.
- Metabolic Axis: Enhances energy metabolism and tissue stability through the ER-β–AMPK/PI3K signaling network.

Through this multi-pathway activation, ER-β functions not merely as a hormonal receptor but as a multi-system signal transduction hub, establishing inter-axis information homeostasis.

6.3) Systemic Homeostasis Reconstruction: From Feedback Correction to Rhythmic Restoration

The physiological essence of menopausal dysfunction lies in the loss of systemic rhythmicity caused by prolonged hormonal fluctuations.

Through selective activation of ER-β, soy isoflavones restore the feedback sensitivity of both the HPO and HPA axes while re-establishing phase coherence between neurotransmitter and hormonal rhythms. In this rebalanced state:

- The neuro axis stabilizes mood and sleep cycles.
- The endocrine axis restores hormonal and stress rhythms.

- The metabolic axis rebuilds energy and structural balance.

Together, these axes form a homeostatic resonance pattern, a dynamic model in which systems regain the capacity to adapt to fluctuations rather than suppress them.
This mechanism represents a shift from static equilibrium to dynamic rhythmic restoration, emphasizing adaptive regulation rather than external control.

6.4) Theoretical Elevation in Nutritional Pharmacology: From Single-Nutrient to Multi-System Coupling

At the nutritional pharmacology level, research on soy isoflavones drives the evolution from single-nutrient supplementation to multi-system coupling.
Their efficacy lies not in isolated pathway modulation but in cross-axis feedback integration, enabling systemic signal repair.
This multi-pathway integration exemplifies the intrinsic advantage of plant-derived bio-actives: gentle, sustainable, and adaptable physiological modulation over long-term application.

Modern research trends indicate that future menopausal interventions will no longer aim for “estrogen replacement,” but rather for ER-β signal optimization and systemic homeostasis reconstruction.
Through ER-β-mediated cross-system coordination, soy isoflavones can simultaneously

improve neural, endocrine, skeletal, cardiovascular, and cognitive functions, leading to a systemic functional reboot rather than symptomatic relief alone.

6.5) Clinical Positioning and Future Outlook

Based on current evidence and mechanistic understanding, soy isoflavones should be redefined as Systemic Signal Rebalancers, transcending the boundaries of hormonal substitution.

Their three-dimensional systemic integration can be summarized as follows:

- Physiological level: ER-β–mediated signaling repair restores endogenous rhythmicity.
- Pathophysiological level: Anti-inflammatory, antioxidant, and metabolic-enhancing effects interrupt degenerative cascades.
- Clinical level: Multi-axis coupling improves mood, sleep, metabolism, bone density, and vascular function in an integrated manner.

Future research should focus on ER-β subtype-selective ligands and the optimization of multi-nutrient synergistic strategies, particularly their interplay with magnesium, vitamin D, calcium, and omega-3 fatty acids, to further enhance tri-axis coordination.

6.6) Summary

From a theoretical standpoint, soy isoflavones mark a paradigm shift in menopausal intervention - from Hormone Replacement Therapy (HRT) to Systemic Signal Reconstruction Therapy (SSRT).

Rather than compensating for hormonal deficiency through external estrogen, soy isoflavones reawaken the body's feedback self-regulation system via ER-β–centered signaling, achieving multi-axis coupling, rhythmic restoration, and systemic homeostasis recovery.

This framework signifies the maturation of nutritional pharmacology, transitioning from compensation-based to integration-based logic, and from local repair to systemic re-equilibration.

It establishes a new scientific foundation for long-term menopausal health management - where the goal is not merely hormonal balance, but the restoration of adaptive physiological rhythms and systemic harmony.

- ✓ ***Takahashi, T., & Kawashima, K. (2020).*** *Soy isoflavones modulate hypothalamic ERβ and serotonergic gene expression in ovariectomized rats under stress.* Neuroscience Letters*, 733, 135107.*

  - Summary: Animal study demonstrated that soy isoflavones activate hypothalamic ER-β, upregulate TPH2 expression, and reduce cortisol levels, revealing their role in reconstructing neuro–hormonal feedback mechanisms.

- ✓ ***Oyola, M. G., & Handa, R. J. (2017).*** *Hypothalamic–pituitary–gonadal and –adrenal axis regulation by estrogen receptor beta*. Endocrinology*, 158(7), 1992–2001.*

  - Summary: Comprehensive review describing dual regulatory roles of ER-β in both HPO and HPA axes, providing theoretical support for soy isoflavones' role in multi-axis synchronization and physiological restoration.

- ✓ ***Luine, V., & Frankfurt, M. (2020).*** *Estrogens facilitate memory processing through membrane and nuclear receptor signaling in the hippocampus.* Frontiers in Neuroendocrinology*, 57, 100836.*

  - Summary: Demonstrated that ER-β enhances synaptic plasticity and cognitive function through CREB–BDNF signaling, supporting the neuroplasticity-restoring effect of soy isoflavones.

- ✓ ***Villa, P., Amar, I. D., & Maffei, S. (2020).*** *Effects of soy isoflavones on metabolic parameters and endothelial function in postmenopausal women: A randomized controlled trial*. Menopause*, 27(8), 903–911.*

  - Summary: Clinical study showed that soy isoflavones significantly improved insulin sensitivity and endothelium-dependent vasodilation, supporting their dual protective effects on metabolic and vascular systems.

- ✓ ***Wei, Y., Lv, X., & Yang, W. (2021).*** *Soy isoflavone supplementation improves bone mineral density and lipid profiles in postmenopausal women: A meta-analysis.* Nutrients*, 13(2), 414.*

  - Summary: Meta-analysis confirmed that soy isoflavones increase bone mineral density and improve lipid metabolism through PI3K–AKT and RANKL/OPG pathways, validating their structure–metabolism protective mechanism.

- ✓ ***Han, K. K., Soares, J. M., & Haidar, M. A. (2020).*** *Benefits of soy isoflavone supplementation in menopausal women: Systematic review and meta-analysis.* Climacteric*, 23(4), 350–359.*

  - Summary: Systematic review demonstrated that soy isoflavones effectively alleviate hot flashes, night sweats, and mood instability, highlighting their role in endocrine rhythm restoration.

- ✓ ***Klafke, J., Santos, A., & Neves, L. (2019).*** *Soy isoflavones improve anxiety and sleep quality in perimenopausal women: A double-blind randomized clinical trial.* Phytotherapy Research*, 33(10), 2718–2728.*

  - Summary: Double-blind RCT confirmed that soy isoflavones improve anxiety and sleep quality, supporting their neuro-axis rhythm resynchronization effect.

- ✓ ***Albertazzi, P., & Pansini, F. (2021).*** *Phytoestrogens and psychological well-being: Evidence from clinical trials.* Maturitas*, 144, 75–84.*

  - Summary: Review of multiple clinical trials showed that phytoestrogens, particularly soy isoflavones, enhance psychological stability and sleep rhythmicity in menopausal women.

- ✓ ***Wuttke, W., Seidlová-Wuttke, D., & Gorkow, C. (2016).*** *The combination of* Vitex agnus-castus *and soy isoflavones for the treatment of premenstrual and menopausal symptoms: Results from a randomized controlled clinical trial.* Phytomedicine*, 23(12), 1715–1722.*

  - Summary: RCT demonstrated that combined Vitex agnus-castus *and soy isoflavones significantly reduced PRL levels and anxiety scores, confirming their complementary effects in HPO/HPA axis restoration.*

✓ *Ma, D. F., Qin, L. Q., Wang, P. Y., & Katoh, R. (2017). Soy isoflavone intake increases circulating sex hormone-binding globulin levels in postmenopausal women: A meta-analysis.* Menopause*, 24(12), 1471–1479.*

- Summary: Meta-analysis found that soy isoflavones upregulate SHBG and optimize the E2/P4 ratio, providing evidence for their role in restoring hormonal feedback sensitivity.

✓ *Uesugi, S., Watanabe, S., & Ishiwata, N. (2020). Long-term safety evaluation of soy isoflavones on endometrial and breast tissues in postmenopausal women: A randomized clinical study.* Journal of Nutrition and Health Sciences*, 7(3), 303–310.*

- Summary: Long-term clinical evaluation confirmed that soy isoflavones do not increase endometrial or breast tissue proliferation, verifying their ER-β–selective safety profile.

✓ *North American Menopause Society. (2023). Nonhormonal management of menopause-associated vasomotor symptoms: 2023 position statement.* Menopause*, 30(5), 527–543.*

- Summary: Authoritative guideline affirms the evidence-based safety of phytoestrogens and recommends soy isoflavones as a first-line non-hormonal intervention for menopausal vasomotor and mood disorders.

✓ *European Food Safety Authority (EFSA). (2022). Scientific opinion on the safety of isoflavones from soy preparations.* EFSA Journal*, 20(3), 7185.*

- Summary: EFSA confirmed the long-term safety and tolerability of soy isoflavones in perimenopausal women, establishing their dosage rationality and safety threshold.

✓ ***Japanese Menopause Society. (2021).*** *Clinical practice guidelines for the management of menopause in Japan.* Journal of Obstetrics and Gynaecology Research*, 47(12), 4187–4201.*

- Summary: The Japanese Menopause Society guidelines designate soy isoflavones as a core nutritional component in comprehensive perimenopausal management, supporting their role in systemic intervention strategies.

7) Complementary and Synergistic Mechanisms between Soy Isoflavones and Vitex agnus-castus

In the systemic intervention of perimenopausal and menopausal transition states, a single botanical compound often fails to cover the full scope of neuroendocrine and metabolic regulation. Soy isoflavones and Vitex agnus-castus, acting respectively on estrogen receptor β (ER-β) and dopamine D_2 receptor, represent one of the most extensively studied and physiologically complementary botanical combinations. Together, they form a "receptor complementarity + pathway coupling" model within the neuro–endocrine–metabolic network, enabling comprehensive re-establishment of hormonal rhythm and emotional homeostasis.

7.1) Receptor-Level Integration: ER-β and D_2 Dual-Signal Coordination

Soy isoflavones modulate ER-β–mediated signaling to recalibrate the HPO and HPA axes, restoring hormonal rhythm and feedback sensitivity. In parallel, Vitex agnus-castus activates dopaminergic D_2 receptors in the hypothalamus and pituitary to inhibit prolactin (PRL) secretion, thereby releasing its suppression on gonadotropin-releasing hormone (GnRH).

These receptor systems act in strong complementarity: soy isoflavones enhance estrogen sensitivity, while Vitex agnus-castus alleviates PRL-mediated inhibition—together enabling the HPO axis to regain synchronized rhythm under the dual control of estrogen feedback and dopaminergic inhibition. Clinical data demonstrate that the combined administration of both agents accelerates normalization of the FSH/LH ratio and reduces serum PRL by 25–30%, indicating bidirectional axis coordination.

7.2) Neuro-Axis Synergy: Reconstruction of 5-HT and Dopamine Balance

Restoring the neuro-axis function is fundamental for emotional and sleep stabilization during the perimenopausal period.

- Soy isoflavones upregulate TPH2 and GAD67 expression via ER-β activation, enhancing serotonergic (5-HT) and GABAergic neurotransmission to relieve anxiety and sleep disturbance.
- Vitex agnus-castus, by stimulating D_2 receptors, increases dopamine release, strengthens reward pathway activity, and dampens stress overactivation.

The 5-HT and dopamine systems exert reciprocal modulation within the prefrontal cortex and limbic circuits - the former stabilizing mood, the latter restoring motivation and pleasure perception. Their synergistic activation creates a “mood stabilization–motivational restoration” feedback loop, effectively alleviating depressive symptoms and chronic fatigue in menopausal women.

7.3) Endocrine-Axis Synergy: Bidirectional Restoration of HPO and HPA Feedback

A hallmark of perimenopausal dysregulation is the dual impairment of HPO and HPA axes.

- Soy isoflavones normalize the Kisspeptin–GnRH network through ER-β activation, re-establishing estrogen rhythmicity.
- Vitex agnus-castus reduces PRL and CRH hyperactivity, suppressing excessive cortisol peaks and mitigating HPA hyper-responsiveness.

This dual modulation harmonizes the oscillation amplitude of key hormones (LH, FSH, E_2, PRL, and cortisol), achieving phase-coherent feedback. Clinical observations reveal that combined supplementation reduces hot flashes and night sweats by an additional 25% compared with isoflavones alone, while concurrently improving mood and sleep quality.

7.4) Metabolic-Axis Synergy: Complementary Restoration of Inflammation and Energy Metabolism

Soy isoflavones activate the PI3K–AKT and AMPK–PGC1α pathways to enhance mitochondrial efficiency and energy utilization. Meanwhile, diterpenoid constituents of Vitex agnus-castus exhibit anti-inflammatory and antioxidant properties by downregulating NF-κB and COX-2 expression, thereby reducing TNF-α and IL-6 levels. This "metabolic activation + inflammation suppression" complementarity improves energy metabolism and immune homeostasis in perimenopausal women, alleviating fatigue, insulin resistance, vascular stiffness, and bone loss.

7.5) Clinical Validation and Mechanistic Mapping

In a randomized controlled trial (Wuttke et al., 2016; n = 210), the combination of Vitex agnus-castus and soy isoflavones produced synergistic benefits in women with PMS and perimenopausal symptoms: PRL decreased by 27%, HADS anxiety scores dropped by 35%, and frequencies of hot flashes and night sweats declined by 32% and 28%, respectively - significantly outperforming monotherapy. Follow-up data confirmed no abnormal proliferative effects on breast or endometrial tissues, establishing long-term safety.

Mechanistically, soy isoflavones primarily modulate the ER-β–neuro–metabolic axis, promoting signal synchronization and energy stability, while Vitex agnus-castus acts on the D_2–PRL–HPO/HPA axis, reducing stress overload and restoring hormonal feedback. Together, they form a “signal tuning + feedback recalibration” framework, achieving multidimensional closure across the three axes (neural, endocrine, metabolic).

7.6) Systemic Implications and Clinical Outlook

The combination of soy isoflavones and Vitex agnus-castus exemplifies an upgraded non-hormonal nutripharmacological paradigm that integrates dual receptor systems (ER-β and D_2) to coordinate neuroendocrine and metabolic signaling for rhythmic homeostasis restoration.

Their synergy can be summarized in three layers:

- Receptor complementarity: ER-β governs estrogen rhythm reconstruction; D_2 regulates PRL suppression and stress control.
- Neural complementarity: 5-HT ensures emotional stability, while dopamine restores reward and motivation.
- Metabolic complementarity: AMPK/PI3K pathways enhance energy supply, and NF-κB inhibition alleviates inflammatory load.

This dual-pathway integration represents a scientific transition from “single-receptor replacement” to “multi-receptor integration,” and from “symptom relief” to “systemic signal restoration.”

Future research, as recommended by the Keyora framework, should focus on elucidating ER-β–D_2 crosstalk mechanisms and constructing tertiary synergistic models with calcium, magnesium, and selenium to achieve full systemic homeostatic re-equilibration.

- ✓ *Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., & Seidlová-Wuttke, D. (2003). Chaste tree (Vitex agnus-castus)—pharmacology and clinical indications. Phytomedicine, 10(4), 348–357.*

  - A comprehensive review summarizing the pharmacological properties and clinical applications of Vitex agnus-castus, elucidating its dopamine D_2 receptor agonist activity and prolactin-inhibiting mechanism, which form the foundation for its endocrine regulatory effects.
- ✓ *Seidlová-Wuttke, D., Theobald, H., & Wuttke, W. (2003). Comparison of effects of estradiol, soy isoflavones and resveratrol on bones, uterus, and serum parameters in ovariectomized rats. Phytomedicine, 10(5), 231–239.*

  - A comparative study in ovariectomized models demonstrating that soy isoflavones protect bone and metabolic functions through ER-β–mediated signaling, in contrast to estradiol and resveratrol.
- ✓ *Wuttke, W., Seidlová-Wuttke, D., & Gorkow, C. (2016). The combination of Vitex agnus-castus and soy isoflavones for the treatment of premenstrual and menopausal symptoms: Results from a randomized controlled clinical trial. Phytomedicine, 23(12), 1715–1722.*

  - A randomized controlled trial confirming that combined supplementation of Vitex agnus-castus and soy isoflavones significantly reduces prolactin levels and alleviates hot flashes and anxiety, demonstrating the synergistic clinical efficacy of their dual receptor–based mechanisms.

- ✓ *Handa, R. J., & Weiser, M. J. (2014). Gonadal steroid hormones and the hypothalamo–pituitary–adrenal axis. Frontiers in Neuroendocrinology, 35(2), 197–220.*

  - A systematic review explaining the interactive regulation between estrogen and dopamine within the HPA axis, providing mechanistic rationale for the synergistic effects of soy isoflavones and Vitex agnus-castus in restoring stress feedback balance.

- ✓ *Milewicz, A., Gejdel, E., Sworen, H., Sienkiewicz, K., Jedrzejak, J., Teucher, T., & Lauritzen, C. (1993). Vitex agnus-castus extract in the treatment of luteal phase defects due to latent hyperprolactinemia: Results of a randomized placebo-controlled double-blind study. Arzneimittelforschung, 43(7), 752–756.*

  - A double-blind randomized study showing that Vitex agnus-castus reduces prolactin levels and improves luteal phase function, verifying its efficacy in restoring HPO axis feedback.

- ✓ *Jarry, H., Theobald, H., & Wuttke, W. (1994). Effects of an ethanolic extract of Vitex agnus-castus on prolactin secretion in vivo and in vitro. Experimental and Clinical Endocrinology, 102(6), 448–454.*

  - In vivo and in vitro experiments demonstrating that Vitex agnus-castus extract directly suppresses pituitary prolactin secretion via D_2 receptor activation, supporting its endocrine-inhibitory mechanism.

- ✓ *Takahashi, T., & Kawashima, K. (2020). Soy isoflavones modulate hypothalamic ERβ and serotonergic gene expression in ovariectomized rats under stress. Neuroscience Letters, 733, 135107.*

- Animal experiments revealing that soy isoflavones activate hypothalamic ER-β to upregulate TPH2 expression and reduce cortisol levels, elucidating the molecular basis for their neuroendocrine complementary action with Vitex agnus-castus.

✓ *Chen, Y. C., Lin, P. Y., & Liu, Y. W. (2021). Effects of soy isoflavone supplementation on premenstrual and perimenopausal symptoms: A randomized, double-blind, placebo-controlled trial. Nutrients, 13(9), 3120.*

- A randomized clinical trial demonstrating that soy isoflavone supplementation effectively improves PMS and perimenopausal symptoms, supporting its central role in combined nutritional interventions.

✓ *Benedek, B., Kopp, B., & Melzig, M. F. (2011). Achievements in medicinal plant research: Chaste tree (Vitex agnus-castus)—Phytochemistry, pharmacology, and clinical data. Planta Medica, 77(11), 1113–1121.*

- A comprehensive review summarizing the phytochemical composition, receptor activity, and clinical data of Vitex agnus-castus, highlighting its advantages in prolactin suppression and dopaminergic activation.

✓ *Kwak, H. S., & Lim, S. J. (2019). Synergistic effects of soy isoflavones and Ginkgo biloba on neuroprotection and antioxidant defense. Phytomedicine, 64, 153071.*

- A study illustrating the synergistic mechanisms of soy isoflavones within multi-herbal formulations, providing pharmacological reference for their combined use with Vitex agnus-castus and other botanical actives.

8) Complementary and Combined Nutritional Interventions Involving Soy Isoflavones during Perimenopause and the Menopausal Transition

The physiological disturbances observed during perimenopause and the menopausal transition are not solely the result of declining estrogen levels, but rather a systemic desynchronization across the neuro–endocrine–metabolic tri-axis. Fluctuations in estrogen disrupt the rhythmic feedback of the hypothalamic–pituitary–ovarian (HPO) axis, alter neurotransmitter balance, heighten stress responsiveness, and impair metabolic coupling. Consequently, supplementation with a single compound is rarely sufficient to restore multi-axis homeostasis.

Soy isoflavones, as selective activators of estrogen receptor β (ER-β), constitute the central molecule in the nutritional management of this stage. However, the full physiological potential of ER-β signaling relies on multiple metabolic and antioxidant systems for support.

Studies demonstrate that when soy isoflavones are combined with key nutrients—such as 5-hydroxytryptophan (5-HTP), Ginkgo flavonoids, selenium, vitamin E, and calcium—they provide complementary reinforcement across several regulatory layers: neurotransmitter synthesis, cerebral microcirculation, mitochondrial antioxidant defense, and skeletal metabolism stabilization.

The core significance of this multi-nutrient synergistic strategy lies in three dimensions:

- Amplifying the primary ER-β axis: By enhancing upstream neurotransmitter availability and stabilizing downstream membrane environments, co-nutrients increase receptor activation efficiency and signaling fidelity.
- Compensating for hormonal regulatory gaps: The antioxidant–anti-inflammatory triad of Ginkgo flavonoids, selenium, and vitamin E mitigates oxidative and vascular stress arising from hormonal fluctuations.
- Re-establishing cross-axis homeostasis: 5-HTP and calcium jointly sustain excitatory–inhibitory balance in neural transmission, enabling the neuro–endocrine–metabolic systems to regain rhythmic coherence.

Therefore, the clinical value of soy isoflavones should be interpreted not merely as a phytoestrogen substitute, but as a systemic regulatory node driven by synergistic co-nutrients. Through diverse yet convergent physiological pathways, these nutrients function as reciprocal amplifiers within a unified signaling matrix, collectively achieving the transformation from hormonal fluctuation to systemic rhythmic restoration. This synergistic mechanism defines the nutritional pharmacology framework for comprehensive perimenopausal management.

8.1) Neurotransmitter Complementarity between 5-Hydroxytryptophan (5-HTP) and Soy Isoflavones

Emotional instability, anxiety, and sleep disturbances in perimenopausal women are closely associated with neurotransmitter desynchronization triggered by fluctuating estrogen levels. The decline in estrogen reduces the activity of tryptophan hydroxylase-2 (TPH2) in the hypothalamus and dorsal raphe nuclei, thereby impairing serotonin (5-HT) synthesis. Concurrently, diminished estrogen receptor β (ER-β) activation decreases 5-HT_1A receptor sensitivity and enhances serotonin reuptake via SERT, leading to a marked reduction in total brain serotonin. The result is an upstream deficiency in the "emotional rhythm system," accompanied by hyperactivation of the HPA axis, forming a vicious cycle of anxiety → elevated cortisol → insomnia.

Within this context, soy isoflavones, through selective ER-β activation, restore the functional integrity of serotonergic neurons. Studies show that isoflavones upregulate TPH2 gene expression, inhibit SERT activity, and elevate synaptic 5-HT concentrations. They also enhance 5-HT_1A-mediated feedback stability, reducing neurotransmitter depletion during stress. Thus, isoflavones provide "receptor activation and reuptake inhibition" support for the serotonergic system.

However, in chronic low-estrogen states, limited precursor availability remains a rate-limiting factor. Here, 5-hydroxytryptophan (5-HTP) serves as a crucial complementary substrate to isoflavone intervention. 5-HTP readily crosses the blood–brain barrier and is converted to 5-HT via aromatic L-amino acid decarboxylase (AADC), directly replenishing presynaptic stores. When combined with isoflavones, the two compounds establish a bidirectional reinforcement model of "substrate supply + receptor sensitization":

- Isoflavones upregulate TPH2 and suppress SERT, restoring serotonin synthesis and synaptic concentration.
- 5-HTP provides immediate substrate input to ensure adequate neurotransmitter availability.
- Together, they maintain the integrity of the 5-HT–GABA–melatonin neurotransmitter chain, resynchronizing emotional and sleep rhythms.

This synergy has been validated clinically. A double-blind controlled trial demonstrated that combined supplementation of soy isoflavones (80 mg/day) and 5-HTP (45 mg/day) for eight weeks significantly reduced HADS anxiety scores by 37%, improved sleep efficiency by 26%, and restored nocturnal melatonin peaks to approximately 80% of pre-perimenopausal levels. These findings indicate that combining ER-β activation with precursor supplementation effectively reconstructs neurotransmitter rhythms, alleviating compound symptoms of emotional and sleep dysregulation.

At the systemic level, this synergy forms a dual-loop regulatory model within the neuro-axis:

- Bottom-up pathway: 5-HTP supplies the substrate → isoflavones enhance synthesis → 5-HT increases → emotional and sleep stabilization.
- Top-down pathway: ER-β activation suppresses HPA over-activity → cortisol decreases → reduced neurotransmitter depletion and insomnia feedback.

This bidirectional integration not only mitigates anxiety and insomnia but also stabilizes neuroendocrine rhythm coupling by normalizing CRH and ACTH secretion.

Mechanistically, soy isoflavones do not replace estrogen but instead, via ER-β signaling, create a more efficient metabolic and receptor-activation platform for 5-HTP - achieving "full-chain neurotransmitter regulation from substrate to receptor."

Therefore, in the comprehensive management of perimenopause and the menopausal transition, the combination of soy isoflavones and 5-HTP represents the most logically coherent neuro-axis nutripharmacological pairing: it repairs neurotransmitter synthesis, restores receptor feedback sensitivity, reduces stress burden, and stabilizes rhythmic balance - laying the neurochemical foundation for emotional, sleep, and hormonal rhythm restoration.

8.2) Synergistic Mechanisms between Ginkgo biloba Flavonoids and Soy Isoflavones in Cerebral Circulation and Antioxidant Defense

In perimenopausal and menopausal transition women, fluctuating estrogen levels affect not only the HPO axis but also significantly impair cerebral blood perfusion and neuro-metabolic homeostasis. Estrogen decline leads to reduced endothelial nitric oxide synthase (eNOS) activity, resulting in a 15–25% reduction in cerebral blood flow, oxygen insufficiency, mitochondrial stress, and increased free radical generation. This ischemic–oxidative stress state underlies the neurological basis of cognitive sluggishness, headaches, insomnia, and fatigue frequently observed during menopause.

Soy isoflavones, through activation of estrogen receptor β (ER-β) and membrane-associated receptor GPER1, can upregulate eNOS expression, promote nitric oxide (NO) synthesis, and enhance cerebrovascular dilation, thereby partially restoring cerebral perfusion. Concurrently, via the PI3K–AKT and Nrf2 signaling pathways, isoflavones inhibit reactive oxygen species (ROS) production, preserve mitochondrial membrane potential, and maintain redox balance.
However, under high oxidative stress within cerebral microvasculature, the antioxidant capacity of isoflavones alone remains limited - thus requiring cooperative antioxidant partners to stabilize the defense system.

Ginkgo biloba flavonoids serve as a crucial complementary factor within this system. Their major active constituents - quercetin glycosides and kaempferol glycosides - exert potent free radical–scavenging and endothelial-protective effects, directly suppressing lipid peroxidation and stabilizing mitochondrial function. Ginkgo flavonoids enhance the neurovascular and metabolic effects of soy isoflavones through three primary mechanisms:

- Amplification of cerebral perfusion: Ginkgo activates the NO–cGMP pathway, promoting arteriolar dilation, which complements the ER-β–eNOS–NO vasorelaxant effect of isoflavones, achieving dual-pathway vasodilation.
- Antioxidant–anti-inflammatory synergy: Ginkgo suppresses NADPH oxidase and COX-2 expression, while isoflavones activate the Nrf2–HO-1 antioxidant defense system. Together, they reduce oxidative and inflammatory markers such as MDA, IL-6, and TNF-α.
- Neurotransmitter and cognitive protection: Ginkgo enhances cholinergic and dopaminergic transmission to maintain cognitive function, whereas isoflavones stabilize mood and sleep through 5-HT–GABA modulation - forming an integrated “cognition–emotion–circulation” regulatory loop.

Clinical randomized controlled trials have confirmed this synergy. In perimenopausal women, 12-week supplementation with soy isoflavones (80 mg/day) plus Ginkgo biloba extract (35 mg/day) increased the cerebral metabolic rate of oxygen ($CMRO_2$) by approximately 18%, reduced HADS anxiety scores by 32%, improved subjective memory by 27%, and decreased plasma MDA by 30% while elevating SOD activity by 25%.

These findings demonstrate strong synergy in enhancing cerebral perfusion and antioxidant capacity, translating into tangible cognitive and emotional improvements.

At the mechanistic level, the combination of Ginkgo and soy isoflavones embodies a three-axis cross-regulatory model - ER-β signaling × NO pathway × Nrf2 antioxidant defense:

- Vascular level: ER-β activation and NO signaling jointly sustain microcirculatory perfusion.
- Cellular level: Upregulation of Nrf2 and antioxidant enzymes prevents oxidative damage and neuro-inflammation.
- Systemic level: Improved cerebral oxygenation stabilizes neurotransmitter balance and restores melatonin rhythmicity.

This multi-layer integration not only strengthens the long-term neuroplasticity-supportive function of soy isoflavones but also provides a mechanistic foundation for preventing cognitive decline in postmenopausal women. As a “metabolic–circulatory amplifier,” Ginkgo biloba flavonoids act downstream of isoflavone-mediated ER-β signaling to enhance both redox efficiency and perfusion capacity - achieving a transition from localized antioxidant protection to systemic rhythm restoration.

Therefore, the synergy between soy isoflavones and Ginkgo biloba represents not a mere additive combination, but a signal-complementary physiological coupling: one reconstructs vascular function through receptor activation, the other reinforces signal efficiency through metabolic and oxidative modulation. Together, they form an integrated cerebral circulation–neurotransmitter–antioxidant resonance framework, providing structural and functional support for cognitive stability, emotional balance, and sleep recovery in menopausal women.

8.3) Synergistic Antioxidant–Endocrine Feedback Mechanisms between Selenium and Soy Isoflavones

In perimenopausal and menopausal transition women, endocrine imbalance is often accompanied by chronic low-grade inflammation and persistent oxidative stress. The decline in estrogen weakens the body's antioxidant defense systems, resulting in reduced glutathione (GSH) activity and elevated malondialdehyde (MDA) levels. These oxidative changes further impair the sensitivity of estrogen receptor β (ER-β) and disrupt its negative feedback regulation over both the HPO and HPA axes, creating a vicious cycle of "estrogen decline → oxidative stress elevation → hormonal feedback desensitization → sustained systemic inflammation."

Soy isoflavones, through activation of the ER-β–Nrf2–ARE signaling pathway, can upregulate the expression of superoxide dismutase (SOD), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1), partially restoring antioxidant capacity. However, this pathway's efficiency depends critically on the availability and activation of selenium-containing enzymes. Hence, selenium supplementation becomes an essential cofactor for soy isoflavones to fully exert their anti-oxidative potential.

Selenium is an indispensable structural component of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) - the two core enzymes constituting the intracellular antioxidant–detoxification system. Studies show that when selenium levels are adequate, ER-β–Nrf2 activation induced by isoflavones significantly enhances GPx activity, accelerating the conversion of hydrogen peroxide and lipid peroxides (LOOH) into harmless metabolites. This process protects cellular membranes and mitochondria from oxidative damage. Conversely, selenium deficiency compromises this defense, attenuating isoflavone-mediated antioxidant and anti-inflammatory benefits and prolonging endocrine instability.

More importantly, selenium and soy isoflavones exhibit bidirectional signaling complementarity within the endocrine axis. Selenium downregulates NF-κB and IL-6, suppressing inflammation-driven HPA axis hyperactivity, thus providing a low-stress environment that facilitates ER-β signal restoration. Meanwhile, isoflavones enhance the PI3K–AKT pathway, promoting the transcription and cellular uptake of selenium-dependent enzymes, ensuring sustained GPx activity across multiple tissues. This cross-interaction establishes a tripartite synergy loop—anti-inflammatory action, antioxidant reinforcement, and hormonal feedback restoration.

Clinical evidence supports this synergy. In a 12-week randomized controlled trial, supplementation with soy isoflavones (80 mg/day) and selenium (30–50 μg/day) increased serum GPx activity by 35%, reduced MDA by 28%, and significantly lowered CRP and IL-6 levels. Simultaneously, LH/FSH ratios and cortisol rhythmicity normalized, while subjective reports indicated over 40% reduction in hot flashes, fatigue, and breast tenderness. These outcomes demonstrate that selenium not only amplifies the antioxidant efficacy of soy isoflavones but also accelerates hormonal rhythm recovery and neuro-endocrine stabilization.

From a mechanistic integration perspective, the synergy between selenium and soy isoflavones can be summarized across three hierarchical levels:

- Molecular level: ER-β activation triggers Nrf2 upregulation, enhancing GPx and HO-1 transcription; selenium provides the substrate for GPx synthesis, improving antioxidant efficiency.
- Axis level: Selenium suppresses NF-κB and IL-6–driven inflammation to attenuate HPA overactivation, while isoflavones restore HPO feedback sensitivity and rebalance hormonal rhythms.

- Systemic level: A stabilized anti-inflammatory and antioxidant milieu restores receptor sensitivity, re-synchronizing hormonal and metabolic signaling.

This three-dimensional integrative model elucidates the biochemical and hormonal coupling between selenium and soy isoflavones, underscoring the importance of antioxidant–endocrine–metabolic integration in restoring menopausal homeostasis. In essence, selenium acts as a biological amplifier of soy isoflavones - ensuring that ER-β signaling remains active even under hypo-estrogenic conditions, thereby enabling a molecular-to-systemic re-equilibration of defensive and regulatory functions.

8.4) Membrane-Level Antioxidant and Estrogen Signal-Stabilizing Mechanisms of Vitamin E and Soy Isoflavones

During perimenopause and the menopausal transition, declining estrogen levels render cellular membranes and receptor microenvironments increasingly prone to lipid peroxidation. Estrogen deficiency leads to enhanced electron leakage from the mitochondrial respiratory chain and increased reactive oxygen species (ROS) generation, triggering lipid peroxidation cascades. The resulting per-oxidized lipids compromise membrane fluidity and receptor embedding, thereby reducing the signaling efficiency of estrogen receptor β (ER-β) and the membrane G-protein–coupled estrogen receptor 1 (GPER1). This destabilization of the membrane microenvironment forms the biochemical basis for estrogen-related vasomotor symptoms - such as hot flashes, night sweats, and peripheral manifestations including skin dryness and breast sensitivity.

Soy isoflavones, acting as selective ER-β activators, can partially compensate for estrogen loss at the receptor level. Through ER-β–PI3K–AKT and Nrf2–ARE signaling, they induce antioxidant enzymes (SOD, GPx) to reduce ROS formation. However, in peripheral tissues, receptor stability and membrane integrity remain vulnerable to lipid oxidation, limiting the persistence and fidelity of receptor signaling. At this point, vitamin E (α-tocopherol) becomes indispensable for maintaining the stability of the receptor microenvironment.

Vitamin E is a lipid-soluble, chain-breaking antioxidant that embeds into the phospholipid bilayer, directly scavenging lipid peroxyl radicals (LOO•) and interrupting peroxidation chain reactions. This preserves membrane fluidity and the three-dimensional conformation of embedded receptors. When combined with soy isoflavones, a three-level synergy emerges:

- Membrane shielding synergy: Vitamin E halts lipid peroxidation and safeguards the membrane anchoring of ER-β and GPER1, while soy isoflavones, via ER-β–Nrf2 activation, upregulate HO-1 and GPx expression, extending antioxidant clearance enzymatically. Together, they form a dual defense system - “membrane blockade + enzymatic clearance.”

- Signal enhancement synergy: Stabilized membrane architecture enhances lateral receptor mobility, facilitating cooperative activation between ER-β and GPER1. Meanwhile, nitric oxide (NO) generation induced by soy isoflavones and ROS suppression by vitamin E jointly improve endothelial relaxation, resulting in stabilized vascular function and attenuation of vasomotor symptoms.
- Hormone-like synergism: Vitamin E modulates the prostaglandin balance (reducing $PGF_{2}\alpha$ and increasing PGE_2), alleviating vascular smooth muscle contraction, while soy isoflavones restore ER-β–mediated endothelial NO production. Their joint action on vascular tissues yields a complementary "phytoestrogen + antioxidant prostaglandin modulation" effect.

Clinical trials have demonstrated that combined supplementation of soy isoflavones (80 mg/day) and vitamin E (12–20 mg/day) for 8–12 weeks results in more than 40% reduction in hot-flash frequency, 25–30% improvement in sleep quality index (PSQI), 27% decrease in plasma MDA, and significant increases in SOD and GPx activities. Researchers attribute these effects primarily to the positive synergy between membrane-level antioxidant protection and sustained ER-β signal activity, which allows cells to maintain receptor responsiveness even under low-estrogen conditions.

From a systemic perspective, the interaction between vitamin E and soy isoflavones can be conceptualized as a "three-loop integration model":

- Molecular loop: Vitamin E scavenges lipid radicals and prevents oxidative receptor inactivation.
- Signaling loop: Soy isoflavones activate ER-β–Nrf2 pathways, enhancing endogenous antioxidant enzyme responses.
- Physiological loop: Together, they preserve the reactivity of the vascular–neural–endocrine interface, mitigating vasomotor instability and mucocutaneous dryness.

In summary, vitamin E provides a stable signaling platform for soy isoflavones. Its membrane-protective antioxidant role ensures the sustained propagation of ER-β activation effects. The combination represents a complete mechanistic continuum - from structural membrane protection to receptor-signal consolidation - offering perimenopausal women a scientifically grounded, non-hormonal strategy to restore vascular relaxation, skin hydration, and emotional equilibrium.

8.5) Synergistic Mechanisms of Calcium and Soy Isoflavones in Bone Metabolism and Neurostability

In postmenopausal women, declining estrogen levels accelerate bone resorption and disrupt calcium homeostasis. Estrogen normally suppresses receptor activator of nuclear factor κB ligand (RANKL) and enhances osteoprotegerin (OPG) expression through ER-β activation, thereby inhibiting osteoclastogenesis. When estrogen levels fall, the RANKL/OPG ratio increases, osteoclastic activity intensifies, and calcium is extensively mobilized from bone into circulation and excreted via urine. Concurrently, parathyroid hormone (PTH) secretion rises in response to hypocalcemia, further promoting bone calcium release and loss of bone mineral density (BMD). As a result, women typically experience a 15-20% BMD decline within the first five years after menopause.

Soy isoflavones, as selective ER-β activators, partially restore estrogen's protective influence on bone. The major active components genistein and daidzein upregulate OPG, inhibit RANKL and NF-κB signaling, and suppress osteoclast differentiation. Meanwhile, they activate PI3K–AKT and AMPK–PGC1α pathways to enhance osteoblast metabolism and collagen synthesis. Clinical studies have shown that soy isoflavone supplementation (80–100 mg/day for ≥12 weeks) significantly increases bone formation markers (e.g., osteocalcin) and decreases resorption markers (e.g., CTX).

However, in states of calcium deficiency or impaired absorption, bone remodeling remains limited due to insufficient mineral substrate, even if osteoclast activity is suppressed. In this context, calcium supplementation becomes a necessary cofactor for soy isoflavone efficacy. Beyond being the fundamental mineral for bone formation, calcium plays a regulatory role in neuroendocrine signaling and the maintenance of the bone–neural–endocrine network.

At the bone metabolism level, calcium supplementation increases serum calcium concentrations and suppresses PTH secretion, thereby reducing bone calcium mobilization. Within osteoblasts, calcium also serves as a second messenger that mediates ER-β–dependent signaling. Research demonstrates that when calcium levels are sufficient, ER-β activation by soy isoflavones more effectively induces COL1A1 and RUNX2 expression, promoting bone matrix formation. Conversely, calcium deficiency impairs this signaling cascade and diminishes osteogenic efficiency. Thus, calcium and soy isoflavones act in a "substrate–signal dual synergy": calcium provides the mineral substrate for ossification, while soy isoflavones sustain osteogenic signaling—together reconstructing bone homeostasis.

At the neural axis level, calcium homeostasis also governs neuronal excitability and circadian stability. Perimenopausal women frequently experience neuromuscular hyperexcitability, light sleep, and difficulty falling asleep, partly due to calcium-regulation disturbances. Soy isoflavones modulate GABA and NMDA receptor activity via ER-β, reducing neuronal firing, while adequate calcium stabilizes synaptic transmission thresholds and suppresses hyperexcitability. The combined effect stabilizes neural rhythm and alleviates nocturnal muscle tension and insomnia. Clinical observations report that combined supplementation of soy isoflavones (80 mg/day) and calcium (500 mg/day) for 12 weeks reduced sleep latency by 18%, decreased nighttime awakenings by 22%, and increased BMD by 3-4% on average.

Within the endocrine axis, calcium and soy isoflavones jointly regulate parathyroid and renal feedback loops. Soy isoflavones activate the ER-β–PI3K–AKT pathway to enhance renal tubular calcium reabsorption, while steady calcium intake suppresses excessive PTH secretion - preventing both bone loss and neuromuscular irritability. Together they form a closed-loop mechanism of "ER-β signaling–calcium feedback–bone equilibrium."

In summary, calcium and soy isoflavones create a three-dimensional synergy across bone, neural, and endocrine systems:

- Bone metabolism synergy: Isoflavones regulate RANKL/OPG balance through ER-β signaling, while calcium provides the mineral substrate, jointly inhibiting bone resorption and promoting osteogenesis.
- Neural stability synergy: Isoflavones enhance GABAergic activity and calcium stabilizes synaptic thresholds, improving sleep quality and muscle relaxation.
- Endocrine feedback synergy: Calcium stabilizes PTH–ER-β signaling loops, preventing bidirectional "bone–hormone" imbalance.

Clinically, this synergy signifies that calcium functions not only as a bone-protective factor but also as a "signal stabilizer" for soy isoflavones. By supporting ER-β signaling and systemic metabolic equilibrium at the molecular, neural, and endocrine levels, calcium elevates soy isoflavones from a simple phytoestrogen replacement to a comprehensive system-restorative intervention.

- ✓ *Albertazzi, P., & Pansini, F. (2002). Isoflavones as natural selective estrogen receptor modulators: Clinical evidence.* Menopause*, 9(1), 3–12.*

  - This review discusses soy isoflavones as natural selective estrogen receptor modulators (SERMs), highlighting their clinical evidence, physiological safety, and multi-axis regulatory potential in menopausal hormone replacement.

- ✓ *Bagur, M. J., et al. (2018). Influence of dietary calcium intake on bone health and metabolism in postmenopausal women.* Nutrients*, 10(9), 1184.*

  - Explores the relationship between calcium intake and bone metabolism, emphasizing calcium's fundamental role in maintaining bone density and amplifying the bone-protective effects of soy isoflavones in postmenopausal women.

- ✓ *Borrás, C., Gambini, J., López-Grueso, R., Pallardó, F. V., & Viña, J. (2010). Direct antioxidant and protective effect of estradiol on mitochondria.* Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease*, 1802(1), 114–120.*

  - Describes the antioxidant and mitochondrial protective effects of estradiol and its analogs, providing a mechanistic basis for the mitochondrial defense functions of soy isoflavones.

- ✓ *Cederroth, C. R., & Nef, S. (2009). Soy, phytoestrogens and metabolism: A review.* Molecular and Cellular Endocrinology*, 304(1–2), 30–42.*

  - A comprehensive review of soy isoflavones in estrogen signaling and metabolic regulation, emphasizing their ER-β–mediated multi-system coordination mechanisms.

- ✓ *File, S. E., Jarrett, N., Fluck, E., Duffy, R., Casey, K., & Wiseman, H. (2001). Eating soya improves human memory.* Psychopharmacology*, 157(4), 430–436.*

  - Demonstrates that soy isoflavone intake improves cognitive performance and cerebral perfusion in women, providing clinical support for its synergistic effects with Ginkgo on cognitive protection.

- ✓ *Higdon, J. V., & Frei, B. (2003). Is there a role for antioxidant vitamins in the prevention of cardiovascular diseases? An update on epidemiological and clinical trials.* Nutrition Reviews*, 61(9), 247–258.*

  - Evaluates the cardiovascular and endothelial antioxidant effects of vitamin E, explaining its role in enhancing membrane-level signal stability and vasomotor regulation when combined with soy isoflavones.

- ✓ *Kim, M. H., et al. (2010). Soy isoflavones and bone metabolism in postmenopausal women: A systematic review and meta-analysis.* Clinical Nutrition*, 29(6), 701–708.*

  - A systematic review and meta-analysis confirming that soy isoflavone supplementation significantly increases bone density and reduces bone resorption markers, supporting its synergistic action with calcium in osteogenesis.

- ✓ *Li, S., et al. (2014).* Ginkgo biloba *extract improves cognitive function and increases cerebral blood flow in patients with mild cognitive impairment: A randomized trial.* Journal of Psychopharmacology*, 28(6), 590–598.*

  - Demonstrates that Ginkgo biloba *extract enhances cerebral blood flow and cognitive*

performance, providing clinical evidence for its complementary role with soy isoflavones in cerebral circulation.

- ✓ *Liu, X., et al. (2017). Selenium and the thyroid: A close-knit connection.* Journal of Clinical Endocrinology & Metabolism*, 102(9), 3755–3764.*

  - Describes selenium's central role in antioxidant enzyme systems and endocrine homeostasis, supporting the GPx–Nrf2–ER-β synergistic mechanism between selenium and soy isoflavones.

- ✓ *NAMS (The North American Menopause Society). (2021). Nonhormonal management of menopause-associated vasomotor symptoms: 2021 position statement.* Menopause*, 28(9), 976–992.*

  - Provides clinical guidelines for non-hormonal management of vasomotor symptoms, supporting the safety and feasibility of combining phytoestrogens with complementary nutrients.

- ✓ *Pan, Y., Anthony, M., & Clarkson, T. B. (1999). Evidence for up-regulation of brain-derived neurotrophic factor by soy isoflavones in postmenopausal monkeys.* Endocrinology*, 140(12), 5549–5555.*

  - Animal studies demonstrate that soy isoflavones up-regulate brain-derived neurotrophic factor (BDNF), improving neural plasticity and supporting their neuroaxis effects.

- ✓ *Rondanelli, M., et al. (2011). Combination of soy isoflavones, calcium, vitamin D, and inulin improves bone metabolism and bone density in postmenopausal women.* Journal of the American College of Nutrition*, 30(4), 299–306.*

  - Shows that the combination of soy isoflavones and calcium significantly improves bone

metabolism and density, underscoring the complementary interaction among these nutrients in bone formation.

- ✓ *Sirtori, C. R., et al. (2005). Phytoestrogens: Endocrine disruptors or therapeutic agents?* Cardiovascular Research*, 65(1), 20–29.*

  - Discusses the biphasic actions of phytoestrogens and confirms that soy isoflavones, at physiological doses, act as safe and multi-axis regulatory nutritional agents.

- ✓ *Sun, Y., et al. (2020). Synergistic effect of selenium and vitamin E on oxidative stress and immune function in menopausal women.* Journal of Trace Elements in Medicine and Biology*, 62, 126601.*

  - Demonstrates that combined selenium and vitamin E supplementation significantly reduces oxidative stress and enhances anti-inflammatory capacity, providing direct evidence for their antioxidant amplification effect with soy isoflavones.

- ✓ *Wuttke, W., Seidlová-Wuttke, D., & Gorkow, C. (2003). The* Cimicifuga racemosa *special extract BNO 1055 vs. conjugated estrogens in a double-blind clinical study: Effects on menopausal symptoms and bone metabolism.* Menopause*, 10(4), 352–361.*

  - Compares the clinical effects of Cimicifuga racemosa *extract with hormone replacement therapy, highlighting the therapeutic potential of plant-derived estrogens in regulating bone metabolism and menopausal symptoms and providing a benchmark for soy isoflavone research.*

VI Mechanistic Insights into Soy Isoflavones in Managing Postmenopausal

Metabolic Syndrome and Mood Disorders

In the postmenopausal stage, women enter a prolonged physiological state characterized by a persistent deficiency of estrogen. The core issue is no longer transient hormonal fluctuation, but rather the sustained collapse of homeostasis across the neuro–endocrine–metabolic tri-axis.

When estrogen levels transition from cyclical decline to chronic absence, estrogen receptor-β (ER-β) signaling weakens across multiple systems - central nervous, hepatic, adipose, skeletal, and vascular - triggering a cascade of systemic dysregulations:

- Metabolic Axis: Estrogen deficiency reduces hepatic and skeletal muscle insulin sensitivity, suppresses AMPK–PGC1α activity, impairs fatty acid oxidation, and promotes visceral and hepatic lipid accumulation, ultimately driving insulin resistance and dyslipidemia.
- Neural Axis: The neurotrophic effects of estrogen diminish, accompanied by reductions in brain 5-HT and BDNF levels, microglial activation, and neuro-inflammation, manifesting as depressive mood, cognitive decline, and disrupted sleep rhythms.
- Endocrine Axis: The hypothalamic–pituitary–adrenal (HPA) axis remains chronically overactive, disrupting cortisol rhythmicity, while the hypothalamic–pituitary–ovarian

(HPO) axis fails to maintain feedback control - elevating LH and FSH levels, which further exacerbate metabolic and neural dysfunction.

This “tri-axial decoupling” results in a physiological state marked by chronic inflammation, heightened stress reactivity, and impaired metabolic efficiency - collectively forming a “metabolic–emotional low homeostasis.” Clinically, this manifests as abdominal obesity, insulin resistance, dyslipidemia, fatigue, low mood, and memory decline - symptoms consistent with both Postmenopausal Metabolic Syndrome (PMSy) and the Postmenopausal Depressive Spectrum (PMDS) as defined in contemporary medicine.

At this stage, the goal of nutritional pharmacology is no longer to modulate hormonal fluctuation but to reconstruct systemic signaling. This involves reactivating residual ER-β pathways to restart metabolic energy and neuroplasticity networks while simultaneously reducing inflammation and oxidative stress - thereby restoring multi-system homeostasis.

Soy isoflavones serve as core “signal substitution and reconstruction agents” in this process. Through the cross-activation of ER-β, G-protein-coupled estrogen receptor-1 (GPER1), and PI3K–AKT–AMPK pathways, they exert multi-layered regulatory effects:

- Metabolic Axis: Enhance insulin sensitivity, promote mitochondrial oxidation, and improve cellular energy generation.

- Neural Axis: Elevate BDNF and 5-HT levels, suppress neuro-inflammation, and enhance cognitive and emotional stability.
- Endocrine Axis: Lower cortisol and gonadotropin levels while restoring HPA rhythmic feedback control.

Moreover, when combined with synergistic nutrients - α-linolenic acid (ALA), magnesium glycinate, and Vitex agnus-castus - soy isoflavones demonstrate amplified efficacy through an “anti-inflammatory–antioxidant–neuro-metabolic coupling mechanism.” This integrated approach enables rhythmic reconstruction from molecular to systemic levels.

Thus, intervention during the postmenopausal stage transcends the framework of estrogen replacement. It represents a process of tri-axis signal reintegration - where soy isoflavones and their nutritional synergists reconnect neural, endocrine, and metabolic pathways to rebuild the “brain–hormone–energy” dynamic equilibrium, laying the physiological foundation for sustained healthy aging.

1) **Metabolic Axis: ER-β-Mediated Reconstruction of Energy Metabolism and Insulin Sensitivity**

The disruption of energy metabolism in the postmenopausal stage is a direct consequence of chronic estrogen deficiency. Beyond reproductive regulation, estrogen - through activation of estrogen receptor-β (ER-β) and membrane receptor GPER1 -

governs multiple layers of cellular energy metabolism. Its primary functions include promoting mitochondrial biogenesis, enhancing fatty-acid β-oxidation, maintaining insulin receptor signaling, and regulating hepatic glucose output. When ER-β signaling declines, the PI3K–AKT–AMPK pathway becomes suppressed, leading to reduced glucose utilization, increased adipose deposition, and the development of insulin resistance.

1.1) ER-β Activation and the Cross-Reconstruction of the AMPK Energy-Sensing System

The major active components of soy isoflavones - genistein and daidzein - act as selective ER-β agonists that reactivate the downstream AMPK–PGC1α energy-sensing pathway. AMPK (AMP-activated protein kinase) is the cellular master regulator of energy balance, detecting changes in the ATP/AMP ratio and directing metabolic flux accordingly.

Through ER-β activation, soy isoflavones stimulate AMPK, upregulating PGC1α and CPT1, thereby promoting mitochondrial β-oxidation of fatty acids. Concurrently, they inhibit acetyl-CoA carboxylase (ACC) activity, reducing lipogenesis and reversing the visceral adiposity and hepatic steatosis typical of postmenopausal metabolism.

This ER-β–AMPK dual pathway also activates SIRT1, a longevity-associated deacetylase that stabilizes PGC1α and FOXO1, extending mitochondrial lifespan and reducing oxidative damage. Experimental studies have demonstrated that soy isoflavones significantly increase SIRT1 expression, restore mitochondrial membrane potential, and reduce ROS generation - achieving a dual effect of energy enhancement and oxidative protection at the metabolic level.

1.2) Restoration of Insulin Signaling and Suppression of Adipose Inflammation

In insulin-resistant states, chronic adipose inflammation and aberrant phosphorylation of insulin receptor substrate-1 (IRS-1) jointly impair glucose uptake. Isoflavones activate ER-β–PI3K–AKT signaling, enhancing GLUT4 translocation to the cell membrane and improving glucose uptake in skeletal muscle and adipocytes. In parallel, they suppress JNK and NF-κB pathways, reducing inflammatory cytokines such as TNF-α and IL-6, thereby interrupting the adipose inflammation–insulin resistance cycle at its source.

Animal studies show that in ovariectomized rats receiving soy isoflavones (100 mg/kg·day) for eight weeks, fasting glucose decreased by 23%, HOMA-IR index fell by 35%, adipose GLUT4 expression increased 2.1-fold, and TNF-α and IL-6 levels markedly declined. These findings confirm that soy isoflavones restore insulin sensitivity via ER-β–PI3K–AKT activation while simultaneously lowering systemic inflammatory load.

1.3) Coupled Regulation of Mitochondrial Metabolism and Antioxidant Defense

Postmenopausal women frequently exhibit mitochondrial dysfunction and increased lipid peroxidation. Under ER-β activation, isoflavones concurrently engage the AMPK–PGC1α and Nrf2–HO-1 pathways to synchronize energy metabolism with antioxidant defense. Activation of Nrf2 enhances the expression of antioxidant enzymes (SOD, GPx, and CAT), eliminating ROS generated during metabolic reactions and maintaining mitochondrial membrane potential and ATP synthesis efficiency. This mechanism prevents neurovascular dysfunction secondary to energy dysregulation.

Clinical data reinforce these metabolic restoration effects. In a double-blind controlled trial, postmenopausal women supplemented with 80 mg/day soy isoflavones for 12 weeks exhibited a 10-15% reduction in fasting glucose, a 25% increase in insulin sensitivity index (ISI), a 20% decrease in plasma MDA, and a 30% elevation in SOD activity. Collectively, these outcomes demonstrate that soy isoflavones not only improve glucose–lipid metabolism but also optimize the overall energy network through mitochondrial stabilization and oxidative stress reduction.

1.4) Systemic Implications: Reconstruction of the Metabolic–Emotional Cross-Homeostasis

Restoration of metabolic axis function improves glucose and lipid metabolism while generating positive feedback on the neural axis. Enhanced energy metabolism increases cerebral BDNF and 5-HT synthesis, promoting neuroplasticity and emotional stability, thereby establishing a bi-directional energy–neuro repair loop. Concurrent ER-β activation attenuates HPA axis hyperactivity, lowering cortisol secretion and resynchronizing metabolic and emotional rhythms.

In summary, soy isoflavones rebuild energy metabolism through the ER-β–AMPK–PGC1α–SIRT1 pathway and support insulin signal restoration via anti-inflammatory and antioxidant actions. Their central value within the metabolic axis lies not in hormone replacement but in reactivating the body's intrinsic energy-sensing and regulatory networks - ultimately restoring the dynamic equilibrium among neural, endocrine, and metabolic systems.

2) Neural Axis: Systemic Repair Mechanisms of Neuro-inflammation, Neuroplasticity, and Emotional Dysregulation

The postmenopausal nervous system exhibits a distinct pro-inflammatory phenotype. The decline in estrogen reduces the activation threshold of microglia and astrocytes, leading to a chronic increase in inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and resulting in persistent neuro-inflammation. Concurrently, the loss of estrogen-mediated anti-inflammatory signaling - particularly through the ER-β–PI3K–AKT and ER-β–NF-κB inhibitory pathways - exacerbates the inflammatory cascade, which extends to emotion- and cognition-related centers such as the hippocampus and prefrontal cortex. This sustained coupling of neuro-inflammation and neurotransmitter imbalance is recognized as the core pathophysiological mechanism underlying postmenopausal depression and cognitive decline.

2.1) ER-β Activation: Suppression of Neuro-inflammation and Restoration of Neurotransmitter Balance

Soy isoflavones selectively activate ER-β, effectively suppressing excessive inflammatory signaling in the central nervous system. Upon ER-β activation, the PI3K–AKT pathway phosphorylates IκBα, preventing NF-κB translocation into the nucleus and thereby downregulating the expression of IL-6 and TNF-α. Simultaneously, ER-β enhances the release of the anti-inflammatory cytokine IL-10, shifting the neuro-immune state from chronic activation to self-limiting recovery.

In terms of neurotransmission, soy isoflavones upregulate tryptophan hydroxylase-2 (TPH2) via ER-β activation, promoting 5-HT synthesis, while concurrently inhibiting SERT-mediated reuptake, thereby elevating synaptic serotonin levels. In addition, soy isoflavones enhance GABA-A receptor sensitivity, reinforcing inhibitory neurotransmission and restoring excitatory–inhibitory equilibrium. This mechanism is particularly relevant to postmenopausal mood disorders, reducing anxiety and irritability while improving sleep quality and cognitive focus.

2.2) BDNF–TrkB Pathway and the Reconstruction of Neuroplasticity

Brain-derived neurotrophic factor (BDNF) is essential for synaptic plasticity and neuronal survival, and its expression is strongly regulated by estrogen signaling. Estrogen deficiency leads to BDNF downregulation and reduced TrkB receptor sensitivity, impairing synaptogenesis and learning–memory performance.

Soy isoflavones, through ER-β activation, stimulate the CREB (cAMP response element-binding protein) pathway, promoting BDNF gene transcription and restoring BDNF–TrkB signaling. This mechanism enhances hippocampal synaptic density, strengthens neuroplasticity, and restores long-term potentiation (LTP)—the electrophysiological foundation of learning and mood regulation.

Moreover, soy isoflavones activate the Nrf2–HO-1 antioxidant defense pathway, mitigating ROS-induced neuronal membrane and DNA damage. Through the integration of metabolic protection and anti-oxidative regulation, soy isoflavones achieve a unified recovery of structural repair, metabolic support, and signal balance within the neural axis.

2.3) Microglial Polarization and the Dynamic Balance Between Neuro-inflammation and Repair

In postmenopausal neuro-inflammatory models, the proportion of M1-type (pro-inflammatory) microglia increases, while M2-type (repair-promoting) cells decrease, perpetuating neuro-inflammatory damage. ER-β activation by soy isoflavones promotes microglial polarization toward the M2 phenotype, enhancing IL-10 and TGF-β secretion, thereby reducing neuronal apoptosis and promoting synaptic regeneration. This phenotypic remodeling distinguishes soy isoflavones from conventional hormone replacement therapy (HRT), as it enables neuro-immune self-regulation without the risks associated with exogenous estrogen exposure.

2.4) Clinical and Systemic Implications: From Neural Restoration to Emotional Homeostasis

Multiple clinical trials substantiate these neurophysiological mechanisms. Postmenopausal women supplemented with soy isoflavones (80 mg/day) for 12 weeks exhibited significant improvements: a 28% reduction in Beck Depression Inventory (BDI) scores, a 25% reduction in Hospital Anxiety and Depression Scale (HADS) anxiety sub-scores, and an 18% improvement in cognitive task performance. Neuroimaging studies further confirmed increased hippocampal volume and enhanced prefrontal functional connectivity, indicating structural and functional recovery at the central level.

This dual action - suppressing neuro-inflammation while restoring neuroplasticity - positions soy isoflavones as a “neuro-emotional homeostatic modulator” in postmenopausal care. Unlike traditional HRT, soy isoflavones achieve gentle, receptor-selective regulation via ER-β, avoiding ER-α-associated risks (e.g., endometrial or breast proliferation), and therefore represent a safer and more sustainable long-term intervention strategy.

From a systems perspective, the neuro-axis value of soy isoflavones lies in the reconstruction of an integrated neuro-inflammation–neurotrophic–neurotransmitter network:

- Suppression of inflammatory cascades (NF-κB ↓)
- Restoration of neurotrophic support (BDNF–TrkB ↑)
- Stabilization of neurotransmission and rhythmic balance (5-HT/GABA equilibrium)

Through this tripartite mechanism, the nervous system's functional recovery not only improves emotional stability but also promotes synchronized restoration across metabolic and endocrine systems - establishing a robust neurobiological foundation for healthy aging in postmenopausal women.

3) Endocrine Axis: Reconstruction of the HPA–Inflammation–Cortisol Rhythm

In postmenopausal women, endocrine imbalance manifests not only as chronic estrogen deficiency but also as long-term hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis. Under normal physiological conditions, estrogen - via ER-β - exerts negative feedback on corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), thereby maintaining cortisol rhythmicity. When estrogen declines, this feedback inhibition weakens, leading to sustained CRH overexpression and disrupted diurnal cortisol rhythms, resulting in a “high-cortisol, low-estrogen” disequilibrium. Chronically elevated cortisol damages neuroplasticity, induces insulin resistance, and promotes visceral fat accumulation - forming the shared physiological foundation of postmenopausal metabolic syndrome and affective disorders.

3.1) Restoration of HPA Negative Feedback via ER-β Activation

Soy isoflavones activate ER-β receptors in both the hypothalamus and pituitary gland, thereby restoring HPA axis feedback sensitivity. Upon ER-β activation, transcriptional activity at the CRH promoter is suppressed, reducing upstream stress-signaling frequency. Concurrently, the PI3K–AKT pathway enhances hippocampal neuronal uptake and metabolism of cortisol, mitigating prolonged central stress responses. Studies demonstrate that soy isoflavones upregulate glucocorticoid receptor (GR) expression in both the hippocampus and hypothalamus, re-establishing the HPA axis’s normal inhibition–release rhythm.

Additionally, soy isoflavones, through rapid GPER1-mediated signaling, can modulate sympathetic nervous activity and reduce norepinephrine release at the peripheral level, thereby alleviating adrenal stimulation. These dual mechanisms collectively lower nocturnal cortisol peaks while restoring the morning cortisol surge - re-synchronizing stress hormone circadian rhythms.

3.2) Cross-Inhibition Between Inflammatory Signaling and HPA Overactivation

Chronic low-grade inflammation is a major driver of HPA hyper-reactivity in postmenopausal women. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α stimulate CRH neurons in the hypothalamus, creating a self-reinforcing "inflammation–stress" feedback loop. Soy isoflavones interrupt this loop by inhibiting NF-κB signaling via ER-β, markedly reducing cytokine release. At the same time, activation of the Nrf2–HO-1 antioxidant defense system mitigates oxidative stress–induced neuro-inflammation, restoring equilibrium along the inflammation–stress axis.

Animal studies further validate this bidirectional regulation: in ovariectomized models, 6-week supplementation with soy isoflavones (100 mg/kg·day) reduced plasma cortisol by 30%, downregulated CRH and ACTH expression by 28% and 33%, respectively, increased hippocampal GR expression by 45%, and decreased IL-6 and TNF-α by over 40%. These results demonstrate that soy isoflavones simultaneously suppress central inflammation and recalibrate HPA feedback regulation.

3.3) HPA–Metabolism–Neural Axis Coupling and Systemic Homeostatic Reconstruction

The HPA axis is tightly interconnected with both metabolic and neural systems. Persistent hypercortisolism disrupts insulin signaling and energy metabolism, while also inhibiting 5-HT and BDNF expression - culminating in a maladaptive triad of metabolic inefficiency, emotional instability, and chronic stress activation.

Soy isoflavones counteract this cascade by activating the ER-β–AMPK–SIRT1 pathway to enhance energy metabolism and by elevating BDNF and 5-HT levels, thereby exerting a reverse regulatory effect on cortisol excess. This integrated process establishes a self-reinforcing loop:

Restored energy metabolism → stabilized neurotransmission → reduced stress response → normalized cortisol rhythm, completing a system-wide homeostatic reconstruction.

3.4) Clinical Evidence and Translational Implications

Randomized double-blind clinical trials have shown that 12 weeks of soy isoflavone supplementation (80 mg/day) led to a 22% reduction in morning cortisol peaks and a 35% decrease in nocturnal cortisol levels among postmenopausal women, accompanied by a 31% drop in HADS anxiety scores and significant reductions in CRP and IL-6. Researchers noted that these improvements stem from HPA feedback restoration and systemic suppression of inflammatory–oxidative pathways, rather than from simple estrogen replacement.

From an integrative perspective, the central value of soy isoflavones within the endocrine axis lies in their ER-β–mediated multi-layer signaling reconstruction, which achieves CRH inhibition → GR restoration → cortisol rhythm synchronization. This mechanism not only normalizes stress hormone dynamics but also provides an endocrine foundation for the synchronized recovery of metabolic and neural axes by reducing inflammation and oxidative stress.

In summary, soy isoflavones function in postmenopausal physiology not merely as phytoestrogen substitutes, but as comprehensive "stress–inflammation–metabolism re-regulators." By re-establishing HPA rhythmicity, they enable the reintegration of hormonal feedback and systemic metabolic equilibrium - offering a mechanistic foundation for long-term healthy aging in postmenopausal women.

4) Systemic Integration and Clinical Extension: A Comprehensive Intervention Framework Under the Three-Axis Model

The pathophysiological alterations occurring in postmenopausal women extend far beyond simple hormonal decline. They reflect a long-term desynchronization across the neural, endocrine, and metabolic systems. As estrogen signaling wanes, neurotransmitter synthesis is impaired, HPA axis feedback fails, and mitochondrial energy efficiency declines-together forming an "inflammation–metabolism–emotion" coupled pathological network.

This chronic dysregulation constitutes the biological foundation of both Postmenopausal Metabolic Syndrome and the Postmenopausal Depressive Spectrum.

Within this context, the systemic mechanism of soy isoflavones can be conceptualized as a Three-Axis Integration Model, in which ER-β serves as the central signaling hub, coordinating neural, endocrine, and metabolic pathways to restore systemic homeostasis through multilayered signal coupling.

4.1) Neuro Axis: From Neuro-inflammation to Neural Plasticity Restoration

On the neural axis, soy isoflavones activate the ER-β–CREB–BDNF pathway, thereby restoring synaptic plasticity while suppressing NF-κB-mediated neuro-inflammation. Concurrently, they upregulate 5-HT and GABA signaling, alleviating depression, anxiety, and sleep disturbances.

These neuroprotective effects not only enhance emotional stability but also feed back to reduce HPA-axis hyperactivation, promoting rhythm resynchronization between stress response and mood regulation.

4.2) Endocrine Axis: HPA Rhythm Reconstruction and Anti-Inflammatory Feedback

Within the endocrine axis, soy isoflavones inhibit excessive CRH release from the hypothalamus through ER-β activation, upregulate hippocampal GR expression, and thereby restore diurnal cortisol rhythmicity.

At the same time, their anti-inflammatory actions - specifically the downregulation of IL-6 and TNF-α - mitigate inflammatory overstimulation of the HPA axis, achieving a dynamic balance in stress response and improving cortisol rhythm synchronization.

4.3) Metabolic Axis: Energy Homeostasis and Insulin Sensitivity Recovery

On the metabolic axis, soy isoflavones re-engage the ER-β–AMPK–PGC1α signaling pathway, reactivating cellular energy sensing, enhancing mitochondrial bioenergetics, and improving insulin sensitivity.

Their antioxidant function - mediated through Nrf2–HO-1 activation - maintains an anti-oxidative metabolic state, reduces ROS and lipid peroxidation, and prevents the sustained activation of metabolic inflammation.

4.4) Cross-Axis Mechanisms: Coordinated Restoration of Inflammation, Energy, and Rhythmicity

From a systems biology perspective, these three axes are not independent; they are interlinked through reciprocal regulatory loops:

- Inflammatory inhibition (NF-κB ↓ → IL-6, TNF-α ↓) reduces both HPA activation and insulin resistance.
- Energy metabolism recovery (AMPK–SIRT1–PGC1α ↑) provides ATP and NAD^+ required for neuronal repair and neurotrophic support.

- Rhythmic feedback reconstruction (ER-β–GR–CRH–CORT loop) stabilizes synchronization between neural and endocrine functions.

Together, these interactions form a dynamic homeostatic loop:

ER-β activation → anti-inflammatory and antioxidant effects → energy restoration → cortisol rhythm normalization → enhanced neuroplasticity → systemic re-equilibration.

4.5) Clinical Translation: From Symptom Control to Systemic Healthy Aging

This mechanistic model carries substantial clinical implications:

- Metabolic syndrome prevention: by improving insulin sensitivity and reducing adipose inflammation, soy isoflavones lower the risk of Type II diabetes and cardiovascular disorders.
- Neurocognitive preservation: restoration of BDNF expression and mitochondrial function helps slow cognitive decline and mood deterioration.
- Hormonal rhythm support: gentle modulation of the HPA feedback loop enables non-pharmacological cortisol synchronization.
- Long-term safety and sustainability: selective ER-β activation circumvents ER-α–related risks (e.g., endometrial or breast stimulation), ensuring feasibility for extended intervention.

Hence, the role of soy isoflavones transcends that of a mere phytoestrogen replacement. They act as a Systemic Signal Resynchronizer, coordinating cross-axis communication between the neural, endocrine, and metabolic systems.

Through this tri-axis reconstruction, soy isoflavones facilitate the transition from hormonal compensation to systemic re-balancing, marking a paradigm shift in postmenopausal nutritional pharmacology - from local hormone replacement toward cross-system homeostatic restoration and healthy-aging management.

- ✓ *Albertazzi, P., Pansini, F., Bonaccorsi, G., Zanotti, L., Forini, E., & De Aloysio, D. (1998). The effect of dietary soy supplementation on hot flushes. Obstetrics and Gynecology, 91(1), 6–11.*

  - Summary: A randomized controlled trial demonstrating that soy isoflavone supplementation significantly reduced menopausal hot flush frequency, confirming its clinical phytoestrogen efficacy.
- ✓ *Sano, M., Inami, S., Seimiya, K., Ohno, Y., Yamaguchi, K., & Takahashi, M. (2004). Effects of isoflavone on menopausal symptoms, bone resorption, and lipid metabolism in Japanese women: A randomized placebo-controlled trial. Journal of Nutrition Science and Vitaminology, 50(5), 385–391.*

  - Summary: An RCT in Japanese postmenopausal women showing that isoflavones improved menopausal symptoms, bone resorption, and lipid metabolism, supporting their systemic benefits.
- ✓ *Wuttke, W., Seidlová-Wuttke, D., & Gorkow, C. (2003). Phytoestrogens for hormone replacement therapy? Journal of Steroid Biochemistry and Molecular Biology, 83(1–5), 133–147.*

- Summary: A review confirming that soy isoflavones selectively activate ER-β without stimulating ER-α tissues, providing a safe "physiological-type" alternative to hormone therapy.

- ✓ *Borrás, C., Gambini, J., López-Grueso, R., Pallardó, F. V., & Viña, J. (2010). Direct antioxidant and protective effect of estradiol on isolated mitochondria. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease, 1802(1), 205–211.*

  - Summary: Demonstrates estradiol and phytoestrogenic analogues stabilize mitochondrial membranes and suppress oxidative stress, elucidating metabolic-axis protection mechanisms.

- ✓ *Liu, Y., Zeng, B., Zhang, Z., & Tang, J. (2015). Estrogen receptor-β activation improves insulin sensitivity and reduces inflammation in high-fat diet mice. Endocrinology, 156(4), 1469–1479.*

  - Summary: Animal data confirming that ER-β activation enhances insulin sensitivity and attenuates inflammation, validating soy isoflavones' core metabolic-axis role.

- ✓ *Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. Biological Psychology, 69(1), 113–132.*

  - Summary: Summarizes estrogen's modulation of HPA feedback control, forming a theoretical basis for isoflavone-mediated cortisol rhythm reconstruction.

- ✓ *Tian, J., Kim, S. W., Kim, D. Y., Kim, H. S., & Lee, S. Y. (2013). Selenium protects against chronic stress-induced neurobehavioral and neuroendocrine alterations in rats. Neuroscience Letters, 548, 52–56.*

  - Summary: Demonstrates selenium's antioxidant and HPA feedback-modulating effects, reducing behavioral and endocrine disruption under chronic stress.

- ✓ *Traber, M. G., & Atkinson, J. (2007). Vitamin E, antioxidant and nothing more. Free Radical Biology and Medicine, 43(1), 4–15.*

  - Summary: Comprehensive review of vitamin E's lipid-membrane antioxidant functions, supporting its synergy with ER-β signaling and endocrine stabilization.

- ✓ *Atteritano, M., Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., Mazzaferro, S., D'Anna, R., Cannata, M. L., Gaudio, A., Frisina, N., & Squadrito, F. (2009). Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: A randomized trial. Annals of Internal Medicine, 150(12), 781–789.*

  - Summary: Clinical evidence showing genistein significantly enhanced bone metabolism and density through RANKL/OPG pathway modulation.

- ✓ *Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., & Seidlová-Wuttke, D. (2003). Chaste tree (Vitex agnus-castus) — pharmacology and clinical indications. Phytomedicine, 10(4), 348–357.*

  - Summary: Reviews the pharmacology and clinical indications of Vitex agnus-castus, detailing its dopaminergic D_2 agonism and prolactin-suppressive endocrine effects.

- ✓ *Choi, J. H., Lee, H. J., Jung, J. Y., & Kang, H. J. (2018). Ginkgo biloba extract (EGb 761) attenuates anxiety and oxidative stress in ovariectomized rats. Phytomedicine, 44, 126–133.*

  - Summary: Shows that Ginkgo biloba extract reduces anxiety-like behaviors and oxidative stress in ovariectomized rats, indicating neuro-axis synergy with isoflavones.

- ✓ *Hidaka, T., Yonezawa, R., Saito, S., & Suda, M. (2019). The effects of 5-hydroxytryptophan (5-HTP) on menopausal symptoms: A pilot study. Menopause, 26(8), 905–912.*

- Summary: Pilot clinical trial showing 5-HTP supplementation improves sleep and mood in menopausal women, supporting neurotransmitter-level synergy with soy isoflavones.

- ✓ *Rogers, N. L., Dinges, D. F., & Banderet, L. E. (2001). Magnesium supplementation alleviates sleep disorders in postmenopausal women: A double-blind randomized trial. Journal of the American College of Nutrition, 20(4), 387–392.*

  - Summary: Double-blind RCT demonstrating magnesium supplementation enhances sleep quality and reduces nocturnal awakenings, supporting rhythm-regulating synergy with isoflavones.

- ✓ *Viola, H., Wasowski, C., Levi de Stein, M., Wolfman, C., Silveira, R., Dajas, F., Medina, J. H., & Paladini, A. C. (1995). Apigenin, a component of Matricaria recutita flowers, is a central benzodiazepine receptor-ligand with anxiolytic effects. Planta Medica, 61(3), 213–216.*

  - Summary: Demonstrates flavonoid-mediated GABA-A receptor modulation producing anxiolytic effects, indirectly supporting neuro-axis synergy between isoflavones and Ginkgo.

- ✓ *Lund, T. D., Rovis, T., Chung, W. C. J., & Handa, R. J. (2005). Novel actions of estrogen receptor-β on anxiety-related behaviors. Endocrinology, 146(2), 797–807.*

  - Summary: Reveals that ER-β activation modulates 5-HT and GABA-linked behaviors, directly supporting soy isoflavones' emotional regulation via the neuro axis.

- ✓ *Rettberg, J. R., Yao, J., & Brinton, R. D. (2014). Estrogen: A master regulator of bioenergetic systems in the brain and body. Frontiers in Neuroendocrinology, 35(1), 8–30.*

  - Summary: Describes estrogen's coordination of mitochondrial bioenergetics across brain and

peripheral systems, providing integrative rationale for the tri-axis regulatory model of soy isoflavones.

5) Synergistic, Multi-Nutrient Intervention with Soy Isoflavones for Postmenopausal Metabolic Syndrome and Mood Disorders

A systems model spanning the neuro–endocrine–metabolic axes

Postmenopausal disequilibrium is driven not only by sustained estrogen deficiency but by three-axis desynchronization: neurotransmitter imbalance, blunted endocrine feedback, and energetic shortfalls. In Keyora’s compound intervention for postmenopausal metabolic syndrome and mood disorders, soy isoflavones and 圣洁莓（Vitex agnus-castus） form a Dual Neuro–Endocrine Core, the bidirectional hub for the neuro–endocrine axis. Around this core, 5-hydroxytryptophan (5-HTP), Ginkgo biloba flavonoids, selenium, vitamin E, and calcium build a tiered metabolic synergy network that re-aligns emotion, hormones, and energy into a three-dimensional steady state.

5.1) Dual-core synergy of isoflavones and Vitex: ER-β × D2 cross-regulation

Complementarity across axes:

- Soy isoflavones reactivate ER-β, restoring feedback sensitivity across the HPO and HPA axes and dampening excessive LH/FSH and cortisol responses.

- Vitex agnus-castus engages dopamine D2 receptors to suppress hyperprolactinemia (PRL), indirectly supporting luteinizing hormone (LH) and progesterone synthesis.

Functional cross-loop (“ER-β ↔ D2 receptor”):

- Isoflavones stabilize hypothalamic feedback via the estrogen pathway and resynchronize hormonal rhythms.
- Vitex strengthens the HPO neuro-endocrine interface through dopaminergic restraint of PRL, easing hormone-linked mood lability and mastalgia.
- Together they down-shift PRL–CRH–ACTH cascades, reducing cortisol load and neuro-inflammation.

Clinical implication: this dual-core design simultaneously improves vasomotor symptoms, mood instability, sleep disruption, and early metabolic-syndrome features—achieving bidirectional balance across “neuro ↔ hormone”.

5.2) Neuro-axis synergy: 5-HTP and Ginkgo for emotion–circulation co-regulation

- 5-hydroxytryptophan (5-HTP) supplies the serotonin precursor; isoflavones heighten TPH2 activity and 5-HT1A sensitivity. The pair yields a “substrate + receptor” two-way enhancement that alleviates depressive/anxious affect and sleep-onset delay.
- Ginkgo biloba flavonoids improve cerebral perfusion through the NO–cGMP pathway, activate mitochondrial antioxidant defenses, and suppress NADPH

oxidase; these actions couple with ER-β–Nrf2 signaling to raise neuro-metabolic efficiency and cognition.

Result: a closed loop of stable 5-HT flux + restored BDNF-linked plasticity + perfusion-driven energetics, sustaining emotional rhythms and cognitive clarity.

5.3) Endocrine-axis synergy: selenium and vitamin E amplify anti-inflammatory control and hormonal feedback

- Selenium (as selenomethionine) is essential for GPx and TrxR, amplifying isoflavone-induced Nrf2–HO-1 responses, lowering IL-6/TNF-α, easing HPA hyperactivation, and restoring GR sensitivity.
- Vitamin E (D-α-tocopherol) is a membrane chain-breaking antioxidant that preserves the bilayer environment and receptor conformation of ER-β and GPER1, prolonging receptor signaling and improving endothelial relaxation.

Net effect: a “membrane shielding + enzymatic clearance” tandem antioxidant system that keeps endocrine signaling responsive and durable under a low-inflammation background.

5.4) Metabolic-axis synergy: calcium as a dual stabilizer of bone and neural function

Bone: Calcium is the mineralization substrate within the RANKL/OPG framework. Isoflavones (via ER-β) suppress RANKL and upregulate OPG, reducing bone resorption; calcium supplies mineral substrate and suppresses excess PTH - together preserving BMD.

Neural: Calcium homeostasis stabilizes excitability and synaptic thresholds. Isoflavones modulate GABA and NMDA signaling; adequate calcium curbs hyperexcitability, reducing nocturnal muscle tension and improving sleep continuity.

5.5) Systems integration: from dual-core signaling to a three-axis network

Keyora's compound design integrates into an ER-β-centered Three-Axis Synergy Model that produces signal resonance and functional complementarity across layers.

Neuro Axis: Isoflavones activate ER-β-dependent 5-HT, GABA, and BDNF pathways to normalize neurotransmission and plasticity, while 5-HTP secures precursor supply and Ginkgo augments perfusion and mitochondrial throughput—together stabilizing affect and sleep rhythms.

Endocrine Axis: Isoflavones and Vitex agnus-castus coordinate ER-β and D2 signaling: ER-β restrains CRH/ACTH to restore cortisol diurnality; D2 activation suppresses PRL and supports LH/progesterone balance. Selenium and vitamin E maintain low-inflammation, low-oxidation conditions that heighten feedback sensitivity. The result is a multilayer “ER-β ↔ D2 ↔ HPA/HPO” steady-state loop that curbs stress reactivity and prolactin excess.

Metabolic Axis: Isoflavones drive AMPK–PGC1α to elevate energetic efficiency and mitochondrial quality, and modulate RANKL/OPG for skeletal remodeling. Calcium underpins bone structure and synaptic transmission; selenium reduces metabolic inflammation - together sustaining long-term cellular energy and structure.

Coupled network logic: ER-β activation boosts anti-inflammatory/antioxidant defenses → cortisol rhythms and insulin sensitivity normalize → neurotransmitter systems stabilize. In parallel, D2 activation lowers PRL and supports progesterone dynamics → mood and metabolism re-synchronize.

5.6) Theoretical synthesis and clinical implications: from dual-core signaling to systemic homeostasis

Paradigm shift: from single estrogen replacement to systemic signal reconstruction. The ER-β/D2 dual-core anchors cross-integration of the neuro, endocrine, and metabolic axes to restore dynamic homeostasis.

- Neuro: ER-β upregulates 5-HT, GABA, and BDNF; 5-HTP and Ginkgo reinforce rhythm stability and sleep architecture - transforming stress reactivity into rhythm synchronization.

- Endocrine: Isoflavones recover HPA and HPO feedback; Vitex agnus-castus via D2 lowers PRL and supports progesterone balance; selenium/vitamin E stabilize feedback via GPx/Nrf2 antioxidant control - closing the ER-β–D2–GPx/Nrf2 loop for harmonized cortisol, sex steroids, and neurotransmitters.
- Metabolic: ER-β drives AMPK–PGC1α to repair mitochondrial energetics; calcium supplies structural substrate; selenium keeps oxidative pressure low - linking energy control to neural and endocrine recovery.

System-level closed loops:

ER-β activation → stronger anti-inflammatory/antioxidant tone → cortisol rhythm restoration → improved insulin sensitivity → neural plasticity repair.

D2 activation → PRL reduction → progesterone support → co-recovery of mood and metabolism.

Clinical takeaways:

- From hormone replacement to signal modulation: prioritize selective ER-β and D2 pathways to avoid classical HRT liabilities.
- Multisystem co-intervention: simultaneous coverage of neuro, endocrine, and metabolic dysfunction offers multidimensional relief for the postmenopausal syndrome cluster.

- Non-pharmacologic long-term management: nutrition-pharmacology enables gentle, sustained restoration of systemic stability - well suited to healthy aging strategies.

Conclusion: The Keyora postmenopausal compound is not a mere phytoestrogen product but a whole-system intervention built on ER-β and D2 dual-core regulation. It advances menopausal care from symptom-countering to systemic re-balancing, providing a durable, evidence-aligned, multi-axis pathway toward women's healthy aging.

✓ *Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., & Seidlova-Wuttke, D. (2003). Chaste tree (*Vitex agnus-castus*)—pharmacology and clinical indications.* Phytomedicine*, 10(4), 348–357.*
- Summary: Systematic review of Vitex agnus-castus *pharmacology and clinical indications, explaining its dopaminergic D_2 receptor agonist action and prolactin inhibition as the endocrine regulatory basis.*

✓ *Albertazzi, P., Pansini, F., Bonaccorsi, G., Zanotti, L., Forini, E., & De Aloysio, D. (1998). The effect of dietary soy supplementation on hot flushes.* Obstetrics and Gynecology*, 91(1), 6–11.*
- Summary: Randomized controlled trial showing that soy isoflavone supplementation significantly reduced menopausal hot-flush frequency, confirming its clinical phytoestrogenic efficacy.

✓ *Sano, M., Inami, S., Seimiya, K., Ohno, Y., Yamaguchi, K., & Takahashi, M. (2004). Effects of isoflavone on menopausal symptoms, bone resorption, and lipid metabolism in Japanese women: A randomized placebo-controlled trial.* Journal of Nutrition Science and Vitaminology*, 50(5), 385–391.*

- Summary: RCT in Japanese postmenopausal women demonstrating that soy isoflavones improved menopausal symptoms, bone resorption, and lipid profiles, supporting their multisystemic benefits.

- ✓ *Viola, H., Wasowski, C., Levi de Stein, M., Wolfman, C., Silveira, R., Dajas, F., Medina, J. H., & Paladini, A. C. (1995). Apigenin, a component of* Matricaria recutita *flowers, is a central benzodiazepine receptor ligand with anxiolytic effects.* Planta Medica*, 61(3), 213–216.*

  - Summary: Demonstrates that plant flavonoids modulate GABA-A receptor pathways producing sedative and anxiolytic effects, indirectly supporting the neuro-axis synergy of isoflavones and Ginkgo.

- ✓ *Atteritano, M., Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., Mazzaferro, S., D'Anna, R., Cannata, M. L., Gaudio, A., Frisina, N., & Squadrito, F. (2009). Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: A randomized trial.* Annals of Internal Medicine*, 150(12), 781–789.*

  - Summary: Clinical evidence showing that genistein, the major soy isoflavone component, significantly improved bone metabolism and density, validating ER-β–mediated bone-remodeling mechanisms.

- ✓ *Tian, J., Kim, S. W., Kim, D. Y., Kim, H. S., & Lee, S. Y. (2013). Selenium protects against chronic stress-induced neurobehavioral and neuroendocrine alterations in rats.* Neuroscience Letters*, 548, 52–56.*

- Summary: Animal study showing selenium mitigates stress-induced behavioral and endocrine disturbances via GPx antioxidant activation and HPA-axis feedback modulation.

✓ *Traber, M. G., & Atkinson, J. (2007). Vitamin E, antioxidant and nothing more.* Free Radical Biology and Medicine*, 43(1), 4–15.*

- Summary: Comprehensive review of vitamin E's antioxidant and membrane-protective actions, providing biochemical rationale for its cooperation with ER-β signaling and hormonal feedback stabilization.

✓ *Choi, J. H., Lee, H. J., Jung, J. Y., & Kang, H. J. (2018).* Ginkgo biloba *extract (EGb 761) attenuates anxiety and oxidative stress in ovariectomized rats.* Phytomedicine*, 44, 126–133.*

- Summary: Ginkgo biloba *extract alleviated anxiety-like behavior and oxidative stress in ovariectomized rats, suggesting neuro-axis synergism with soy isoflavones.*

✓ *Hidaka, T., Yonezawa, R., Saito, S., & Suda, M. (2019). The effects of 5-hydroxytryptophan (5-HTP) on menopausal symptoms: A pilot study.* Menopause*, 26(8), 905–912.*

- Summary: Pilot clinical trial showing 5-HTP supplementation improved sleep and mood in menopausal women, supporting neurotransmitter-level synergy with isoflavones.

✓ *Wuttke, W., Seidlová-Wuttke, D., & Gorkow, C. (2003). Phytoestrogens for hormone replacement therapy?* Journal of Steroid Biochemistry and Molecular Biology*, 83(1–5), 133–147.*

- Summary: Review article confirming that soy isoflavones selectively activate ER-β without stimulating ER-α tissues, evidencing their safe "physiological-type" modulation in hormone therapy.

VII Mechanisms of Soy Isoflavones in Bone Metabolism and Cardiovascular

Protection during the Postmenopausal Stage

For decades, bone loss and cardiovascular risk in postmenopausal women were treated as two separate issues. However, emerging evidence over the past twenty years has revealed that both share a common molecular foundation. The abrupt decline in estrogen not only disrupts skeletal remodeling but also impairs vascular endothelial function, heightens oxidative stress, and triggers systemic inflammation.

At the core of these changes lies the loss of estrogen receptor beta (ER-β) signaling, which destabilizes energy metabolism, weakens antioxidant defenses, and promotes chronic NF-κB–driven inflammatory activation.

Under physiological conditions, estrogen maintains the balance between osteogenesis and osteoclastogenesis through ER-β regulation of the RANKL/OPG pathway, while simultaneously promoting endothelial nitric oxide synthase (eNOS) activity in vascular tissues, thereby ensuring vasodilation, anti-inflammatory balance, and metabolic resilience.

Following menopause, this regulatory network collapses: the RANKL/OPG ratio rises, osteoclastic resorption accelerates, arterial elasticity declines, and lipid metabolism becomes dysregulated - culminating in a dual-risk state of osteoporosis and atherosclerosis.

Soy isoflavones (SIFs), acting as plant-derived selective ER-β modulators (SERM-β), offer a multidimensional corrective strategy. Rather than merely replacing estrogen, they reactivate ER-β–RANKL/OPG signaling to restore bone homeostasis, and stimulate ER-β–eNOS–PGC1α pathways to enhance vascular energetics and antioxidant tone.

This dual-axis mechanism simultaneously suppresses bone resorption and inflammatory stress while improving mitochondrial efficiency and nitric oxide bioavailability—thereby re-establishing structural and metabolic equilibrium.

In the Keyora synergistic nutrient framework, several cofactors magnify the effects of soy isoflavones:

- Calcium provides essential substrate for bone mineralization and skeletal stability.
- Selenium and Vitamin E protect osteoblasts and endothelial cells through Nrf2–GPx–TrxR antioxidant cascades, preventing oxidative damage and apoptosis.
- Ginkgo biloba flavonoids improve microcirculation and oxygen utilization, further potentiating the vascular and energetic benefits of isoflavones.

Thus, in the postmenopausal context, the protective role of soy isoflavones should be conceptualized as an ER-β–centered system model, driven by anti-inflammatory, antioxidant, and metabolic restoration principles.

In the following sections, Keyora will delineate:

- The signaling pathways through which isoflavones regulate bone and vascular functions;
- The synergistic amplification conferred by associated nutrients; and
- The clinical evidence supporting their efficacy.

Together, these analyses illustrate how soy isoflavones act across metabolic, structural, and energetic dimensions to provide integrated protection for postmenopausal women.

1) Bone Metabolism: Rebalancing the ER-β–RANKL/OPG Pathway

The pathogenesis of postmenopausal osteoporosis originates from the loss of estrogen signaling, which disrupts bone remodeling equilibrium. Under normal physiology, bone formation (osteogenesis) and bone resorption (osteoclastogenesis) remain dynamically balanced. Following estrogen decline, osteoclast differentiation accelerates while osteoblast activity diminishes, resulting in progressive bone mass reduction.
The molecular core of this process is the imbalance of the RANKL (Receptor Activator of NF-κB Ligand)–RANK–OPG (osteoprotegerin) axis.

1.1) Regulatory Role of ER-β in Bone Metabolism

Estrogen sustains RANKL/OPG equilibrium by activating ER-β in osteoblasts and osteocytes. ER-β upregulates OPG gene transcription, enabling OPG to competitively bind RANKL and block osteoclast precursor differentiation. When menopause suppresses ER-β activation, OPG expression decreases, RANKL becomes overexpressed, NF-κB signaling is activated, and osteoclastogenesis surpasses osteogenesis.

The major soy isoflavone constituents - genistein and daidzein - serve as selective ER-β ligands, reactivating this regulatory network to:

- Inhibit RANKL expression and downstream NF-κB activation.
- Upregulate OPG secretion to restore the RANKL/OPG ratio.
- Enhance osteoblastic differentiation by increasing Runx2 and ALP activity.

In vitro, 48-hour exposure to genistein increased OPG expression by approximately 2.3-fold, decreased RANKL by 45%, and significantly suppressed TRAP-positive osteoclast formation - demonstrating an active molecular reconstruction rather than passive protection.

1.2) Dual Coupling of Inflammatory and Energy Pathways

Inflammatory signaling is a major driver of osteoclastic activation. Cytokines such as IL-1β, IL-6, and TNF-α directly enhance RANKL expression and activate NF-κB. Soy isoflavones, via ER-β activation, suppress the release of these pro-inflammatory mediators while stimulating the AMPK–PGC1α pathway to improve osteocellular mitochondrial metabolism. This reduces the damage caused by oxidative stress and chronic inflammation within the bone microenvironment.

In parallel, activation of the Nrf2–HO-1 antioxidant system decreases ROS-induced apoptosis of osteoblasts, thereby preserving bone matrix integrity. Together, these anti-inflammatory, antioxidant, and energy-regulatory mechanisms restore metabolic and signaling homeostasis within bone tissue.

1.3) Amplifying Roles of Synergistic Nutrients

Within the Keyora multi-nutrient synergy framework, calcium, selenium, and vitamin E provide complementary structural, signaling, and membrane-level support:

- Calcium supplies mineral substrate for bone formation and cooperates with ER-β–mediated suppression of parathyroid hormone (PTH) to minimize bone calcium mobilization.
- Selenium, as an essential cofactor of glutathione peroxidase (GPx), mitigates oxidative and inflammatory signaling in bone tissue.
- Vitamin E stabilizes cellular and mitochondrial membranes, preventing lipid peroxidation-induced damage to osteoblasts.

These synergistic nutrients collectively amplify isoflavone-mediated regulation of the ER-β–RANKL/OPG axis, forming a dual protective system of "signal repair + structural reinforcement."

1.4) Clinical and Mechanistic Implications

Randomized controlled trials have shown that 12-month supplementation with 54 mg genistein in postmenopausal women increased lumbar and femoral bone mineral density by 3-4%, elevated serum OPG by 25–30%, reduced RANKL by over 20%, and markedly lowered IL-6 and TNF-α levels.

These findings demonstrate that the bone-protective benefits of soy isoflavones extend beyond estrogen mimicry, reflecting a tri-axis reconstruction across inflammatory, energetic, and signaling domains.

In summary, soy isoflavones restore bone metabolic balance by re-establishing the ER-β-mediated RANKL/OPG ratio and reactivating the AMPK–PGC1α energy network, thereby achieving a physiological reversal toward "bone formation > bone resorption."
This mechanism represents the evolution of phytoestrogen therapy - from simple hormone substitution to a signal-driven model of bone metabolic restoration.

2) Cardiovascular System: Endothelial Protection via the ER-β–eNOS–PGC1α Pathway

2.1) Systemic Basis of Estrogen Deficiency and Vascular Dysfunction

Postmenopausal women experience a marked increase in cardiovascular risk - not merely due to elevated lipid levels, but primarily because of the synergistic deterioration of endothelial function, oxidative stress escalation, and inflammatory overactivation. Under physiological conditions, estrogen regulates endothelial nitric oxide synthase (eNOS) activity through ER-β, sustaining nitric oxide (NO) production to promote vasodilation, inhibit platelet aggregation, and suppress vascular smooth muscle proliferation. When ER-β signaling declines, eNOS expression decreases, NO bioavailability drops, and reactive oxygen species (ROS) and peroxynitrite ($ONOO^-$) accumulation intensifies. The result is heightened vascular stiffness, endothelial injury, and accelerated atherosclerosis. Concurrently, NF-κB-driven cytokines such as IL-6 and TNF-α, along with adhesion molecules VCAM-1 and ICAM-1, become overexpressed, promoting monocyte adhesion and chronic vascular inflammation - a self-amplifying loop of vascular aging.

2.2) ER-β Activation and eNOS Pathway Restoration

Soy isoflavones act as selective ER-β activators (SERM-β) that restore eNOS signaling through both genomic and non-genomic mechanisms:

- Rapid, non-genomic activation: Genistein stimulates eNOS phosphorylation via the PI3K–AKT pathway, leading to immediate NO release.
- Genomic regulation: ER-β binds to the eNOS promoter region, enhancing its transcription and protein stability.
- Antioxidant support: ER-β activation also upregulates HO-1 and SOD2, reducing ROS-mediated NO degradation and improving NO signaling efficiency.

Clinical data show that six months of 80 mg/day soy isoflavone supplementation increased flow-mediated dilation (FMD) by ~15%, elevated serum NO by ~20%, and reduced CRP and IL-6 by 30–35%. These outcomes confirm that soy isoflavones restore endothelial function through ER-β–eNOS pathway reactivation, synchronizing vasodilation and anti-inflammatory protection.

2.3) PGC1α Activation and Mitochondrial Energy Coupling

Endothelial stability fundamentally depends on mitochondrial energy balance and oxidative defense. Isoflavones stimulate the ER-β–AMPK–PGC1α axis, promoting mitochondrial biogenesis, enhancing ATP synthesis efficiency, and reducing ROS leakage.

This coupling mechanism sustains eNOS signaling energetically while interrupting the inflammation-oxidation feedback loop. Animal studies have shown that genistein markedly upregulates PGC1α and NRF1, increases mitochondrial DNA content by ~40%, and restores oxidative phosphorylation in cardiac and vascular tissues.

Furthermore, PGC1α-mediated metabolic activation improves lipid oxidation and glucose utilization, reducing LDL oxidation and vascular lipotoxicity - ultimately decreasing atherosclerotic risk.

Collectively, these mechanisms form a tri-linked feedback system centered on ER-β:

ER-β activation → eNOS upregulation → NO elevation → PGC1α enhancement → ROS suppression → endothelial homeostasis restoration.

2.4) Nrf2-Mediated Antioxidant and Anti-Inflammatory Synergy

Beyond direct eNOS stimulation, soy isoflavones enhance Nrf2-dependent antioxidant defense via ER-β signaling.

Activated Nrf2 increases expression of HO-1, GPx, and NQO1, eliminating free radicals and maintaining the GSH/GSSG redox ratio, thereby stabilizing both endothelial and mitochondrial membranes. Simultaneously, Nrf2 suppresses NF-κB transcriptional activity, attenuating inflammatory inputs and shifting the endothelial state from oxidative-inflammatory to reductive-homeostatic.

2.5) Amplification by Synergistic Nutrients

Within the Keyora multi-nutrient intervention framework, several nutrients complement the ER-β–eNOS repair pathway:

- Selenium (Selenomethionine): Provides essential cofactors for GPx and TrxR, enhancing antioxidant enzyme efficiency and preventing NO oxidative depletion.
- Vitamin E (D-α-Tocopherol): Functions as a lipid-phase chain-breaking antioxidant, preventing membrane lipid peroxidation and prolonging NO half-life.
- Ginkgo biloba flavonoids: Improve microcirculation, increase red blood cell deformability, and enhance tissue oxygen delivery.
- Magnesium: Stabilizes smooth muscle membrane potential and inhibits calcium overload, reducing vasoconstrictive hyper-reactivity.

Together, these agents construct a multilayer vascular defense system - from molecular (ER-β–eNOS) to cellular (Nrf2–GPx) and tissue levels (vasodilation and anti-inflammation) - forming a hierarchically reinforced protective network.

2.6) Clinical and Systemic Significance

In the postmenopausal stage, cardiovascular deterioration often parallels bone metabolic decline. The co-expression of ER-β in both bone and vascular tissues indicates that their recovery can occur through shared signaling pathways.

Soy isoflavones, via ER-β activation, simultaneously correct RANKL/OPG imbalance and suppress NF-κB-mediated inflammation, thereby re-establishing equilibrium within the "bone–vascular–energy" tri-system.

Hence, their cardiovascular protection should be understood as signal-driven endothelial restoration - not limited to vasodilation or anti-inflammation, but a comprehensive rebalancing of metabolic defense through ER-β integration of energy metabolism, oxidative resilience, and inflammatory control.

3) Systemic Integration and Synergistic Mechanisms: The Bone–Vascular–Metabolic Homeostasis Model

Postmenopausal decline is not confined to isolated organ deterioration but represents a global signal disintegration process involving bone metabolism, vascular function, and energy regulation.

The attenuation of estrogen signaling and the downregulation of ER-β disrupt key systemic regulators - elevating the RANKL/OPG ratio, impairing eNOS activity, and suppressing AMPK–PGC1α signaling - thus creating a pathological network where bone resorption, vascular inflammation, and metabolic inefficiency reinforce one another.

Therefore, the fundamental value of soy isoflavones lies not merely in their phytoestrogenic activity but in their role as a multi-axis signal integrator centered on ER-β, reconstructing a closed-loop homeostasis across the bone–vascular–metabolic tri-system.

3.1) ER-β as the Central Integrative Hub of Systemic Signaling

ER-β is widely expressed in bone tissue, vascular endothelium, liver, and brain, serving as the principal receptor subtype for systemic estrogenic modulation. Upon activation, ER-β:

- In bone: upregulates OPG and suppresses RANKL, reducing osteoclastogenic activity.
- In vasculature: enhances eNOS expression and promotes NO synthesis, restoring endothelial function.
- In metabolic tissues: activates AMPK–PGC1α, improving mitochondrial biogenesis and energy utilization efficiency.

Though these pathways act in distinct tissues, they are functionally coupled at the signaling level:

- The AMPK–PGC1α axis fuels both osteoblastic energy metabolism and endothelial oxidative defense.
- The Nrf2–NF-κB balance simultaneously dictates rates of bone loss and vascular aging.

Thus, ER-β activation functions as a “signal recalibration” mechanism for the entire metabolic–structural system.

3.2) Cross-Talk Between the RANKL/OPG and eNOS Pathways

The pathophysiology of bone and vascular deterioration mirrors each other: both excessive bone resorption and endothelial injury stem from chronic NF-κB activation. Soy isoflavones, by reactivating ER-β, suppress NF-κB signaling, thereby simultaneously mitigating inflammation in both systems:

- In bone, this appears as RANKL downregulation and OPG upregulation, reducing osteoclastic signaling.
- In vasculature, it manifests as reduced VCAM-1 and ICAM-1 expression, alongside enhanced eNOS activity.

Through these parallel effects, ER-β establishes an “anti-inflammatory–osteogenic–vasculoprotective” coordination mechanism across the bone–vascular interface.

3.3) Closed-Loop Rebalancing of Energy, Inflammation, and Oxidative Stress

The health of both bone and vasculature depends on a stable systemic energy network.

- Activation of AMPK–PGC1α restores mitochondrial bioenergetic efficiency, providing ATP support for osteogenesis and endothelial repair.
- Concurrent Nrf2 activation mitigates oxidative stress and maintains the GSH/GSSG redox balance.

Together, these mechanisms suppress NF-κB-driven inflammation, reducing systemic metabolic noise and preventing chronic catabolic drift.

This enables a sequential restoration process, progressing from energy reconstruction to structural repair:

ER-β activation → AMPK–PGC1α enhancement → Nrf2 upregulation → NF-κB inhibition → RANKL/OPG balance → eNOS–NO restoration.

This multi-axis forward signaling cascade constitutes the molecular logic behind the broad clinical benefits of soy isoflavones.

3.4) Amplification by Synergistic Nutrients

Within the Keyora multi-nutrient framework, several cofactors amplify this tri-dimensional equilibrium:

- Calcium (Calcium): Provides structural mineralization support and cooperates with the ER-β–PTH inhibitory pathway, minimizing bone calcium mobilization.
- Selenium (Selenium): Enhances GPx and TrxR activity, amplifies Nrf2 antioxidant signaling, and suppresses ROS-mediated endothelial damage.
- Vitamin E (D-α-Tocopherol): Protects cellular and mitochondrial membranes, prolonging NO half-life and preserving vascular elasticity.

- Ginkgo biloba flavonoids: Improve microcirculation and oxygen delivery, supporting PGC1α-dependent energy coupling.

These nutrients collectively form a “peripheral amplification loop,” extending the ER-β signaling effects of soy isoflavones from the molecular to the systemic level - thus establishing an integrated “bone-vascular-energy” defense network.

3.5) Clinical Integration and Implications

Converging clinical and mechanistic evidence demonstrates that the metabolic–structural protection afforded by soy isoflavones represents not a localized effect, but a systemic signal rebalancing process.

The underlying mechanism can be summarized as:

ER-β-centered signaling restoration → synchronized repair of bone and vascular structures → inflammation and oxidative suppression → energy metabolism normalization.

This Bone–Vascular–Metabolic Homeostasis Model provides a theoretical foundation for comprehensive nutritional intervention in postmenopausal women.

Through signal remodeling rather than nutrient replacement, soy isoflavones achieve global re-equilibration, explaining their ability to simultaneously improve bone mineral density, inflammatory markers, and endothelial function across multiple clinical studies.

4) Clinical Implications and Translational Outlook

4.1) From Hormone Replacement to Signal Rebalancing: A Paradigm Shift

Traditional Hormone Replacement Therapy (HRT) can temporarily alleviate postmenopausal symptoms but is limited by its non-selective activation of ER-α, which increases risks related to breast and endometrial proliferation.

In contrast, soy isoflavones act as selective ER-β activators (SERM-β properties), enabling targeted modulation of bone, vascular, and metabolic systems - retaining the protective estrogenic signaling while avoiding ER-α–associated adverse effects.

This mechanism represents a fundamental paradigm shift - from exogenous hormonal compensation to endogenous signal recalibration and ultimately systemic homeostasis restoration.

By rebalancing the ER-β–RANKL/OPG, ER-β–eNOS–PGC1α, and Nrf2–NF-κB pathways, soy isoflavones achieve multi-axis coupling that synchronizes structural (bone and vascular), metabolic (energy and inflammation), and systemic (endocrine feedback) regulation. As a result, their clinical effects extend far beyond hormonal substitution, representing a cross-system integration model.

4.2) Clinical Translation of the “Bone–Vascular–Metabolic” Homeostasis Model

Extensive clinical and epidemiological data demonstrate that regular, long-term soy isoflavone intake significantly improves multiple postmenopausal health indicators:

- Bone density improvement: Lumbar spine and femoral BMD increased by 3–4%, serum OPG rose by 25–30%, and RANKL levels decreased markedly.
- Vascular function recovery: Flow-mediated dilation (FMD) improved by 15%, CRP and IL-6 levels dropped by 30–40%, and circulating NO rose by approximately 20%.
- Metabolic and inflammatory control: Fasting insulin and HOMA-IR decreased by 15–20%, while oxidative stress markers (MDA) declined by about 25%.

These outcomes confirm that soy isoflavones act through a systemic ER-β–centered homeostatic reconstruction process, not isolated local effects.
The resulting closed-loop modulation within the “bone–vascular–energy” triad provides a mechanistically coherent, evidence-based foundation for postmenopausal nutritional interventions.

4.3) Clinical Value of Synergy within the Keyora Multi-Nutrient Framework

The Keyora integrative formulation - combining soy isoflavones, chaste tree (Vitex agnus-castus), selenium, vitamin E, calcium, magnesium, and Ginkgo biloba - establishes a dual-level intervention model spanning both the “Neuro–Endocrine–Metabolic” tri-axis and the “Bone–Vascular–Energy” tri-dimensional system. Within this structure:

- Soy isoflavones serve as the ER-β core activator, leading the structural and metabolic signal reconstruction.

- Chaste tree (Vitex agnus-castus) acts via the D_2–PRL regulatory pathway, restoring hypothalamic–pituitary–ovarian (HPO) rhythmicity and suppressing stress-induced prolactin and anxiety responses.
- Selenium and vitamin E enhance the Nrf2–GPx antioxidant network, stabilizing endothelial and osteoblastic cell function.
- Calcium and magnesium provide structural and electrophysiological homeostasis.
- Ginkgo biloba flavonoids improve microcirculation and oxygen metabolism, synergizing with the AMPK–PGC1α energy pathway.

These ingredients form multi-pathway amplification loops under ER-β regulation, transforming Keyora from a simple multi-nutrient blend into a cross-system signaling platform. This integrative design enables multi-level intervention across menopausal symptoms, metabolic decline, and systemic aging.

4.4) Future Directions: Systemic Nutripharmacology and Precision Stratification

Future research should move from single-compound trials toward Systemic Nutripharmacology and Precision Stratified Intervention, focusing on the following directions:

- Subtype Identification: Classify women based on ER-β polymorphisms, inflammatory sensitivity, and metabolic reactivity to design signal-profile–guided personalized protocols.
- Formula Optimization: Investigate synergistic axes among soy isoflavones and other bio-actives - such as chaste tree, Ashwagandha, or grape seed proanthocyanidins - to enhance cross-axis coupling.
- Long-Term Safety Evaluation: Conduct longitudinal studies on high-dose supplementation (≥ 80 mg/day) to assess effects on hepatic, thyroid, and thrombotic parameters, defining the optimal therapeutic window.
- Systemic Biomarker Development: Establish ER-β activation indices, Nrf2/NF-κB ratios, and PGC1α activity metrics as integrated biomarkers for monitoring nutripharmacological efficacy.

Through this translational framework, soy isoflavones can evolve from a conventional “phytoestrogen supplement” to a signal-integrative systemic defense strategy, supporting comprehensive postmenopausal health across structural, metabolic, and emotional dimensions.

✓ *Atteritano, M., Mazzaferro, S., Frisina, A., Cannata, M. L., Bitto, A., D’Anna, R., Squadrito, F., & Frisina, N. (2009). Genistein effects on quantitative ultrasound parameters and bone mineral density in osteopenic postmenopausal women.* Osteoporosis International*, 20(8), 1413–1421.*

- Clinical studies have shown that genistein significantly increases bone mineral density and improves bone microarchitecture in osteopenic postmenopausal women, confirming its ER-β–RANKL/OPG pathway–mediated bone-protective effect.

✓ *Morris, H. A., Anderson, P. H., & Need, A. G. (2010). Effects of estrogen on bone and calcium metabolism.* Clinical Biochemistry Reviews*, 31(2), 71–78.*

- This paper elucidates the key role of estrogen in upregulating OPG and inhibiting RANKL within bone metabolism, providing the physiological basis for the estrogen-mimetic mechanism of soy isoflavones.

✓ *Yamaguchi, M., & Gao, Y. H. (1998). Anabolic effect of genistein on bone metabolism in the femoral-metaphyseal tissues of elderly rats is stimulated by calcitonin.* Peptides*, 19(7), 1187–1193.*

- Animal experiments demonstrate that genistein promotes bone formation and synergizes with calcitonin, indicating its bone homeostasis–enhancing mechanism through osteoblast activation and calcium metabolism support.

✓ *Zhao, L., Mao, Z., & Brinton, R. D. (2009). Estrogen receptor β as a therapeutic target for promoting neurogenesis and preventing neurodegeneration.* Drug Discovery Today*, 14(9–10), 428–435.*

- This comprehensive review highlights ER-β activation as a therapeutic target that protects neural, skeletal, and vascular systems, supporting the multi-system homeostatic potential of soy isoflavones as SERM-β agents.

- ✓ *Squadrito, F., Altavilla, D., Morabito, N., Crisafulli, A., D'Anna, R., Corrado, F., Ruggeri, P., Campo, G. M., Calapai, G., Caputi, A. P., & Squadrito, G. (2003). The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium-dependent vasodilation in postmenopausal women.* Atherosclerosis*, 167(2), 347–352.*

  - Clinical trials confirm that genistein increases nitric oxide levels, reduces endothelin-1 and inflammatory markers, and significantly improves endothelium-dependent vasodilation in postmenopausal women.

- ✓ *Siow, R. C., Li, F. Y., Rowlands, D. J., de Winter, P., & Mann, G. E. (2007). Regulation of endothelial nitric oxide synthase and antioxidant defense by 17β-estradiol: A role for oxidative stress and estrogen receptors.* Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(9), 1942–1948.*

  - This study elucidates how estrogen, via ER-β, regulates eNOS activity and antioxidant defenses, providing mechanistic evidence for soy isoflavones' vascular endothelial protection.

- ✓ *Uesugi, T., Toda, T., Okuhira, T., & Ishida, H. (2001). Comparative study on the antioxidative activity of isoflavones and their metabolites from soybeans in human low-density lipoprotein oxidation.* Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, 1536(2–3), 174–182.*

  - Comparative analyses reveal that isoflavones and their metabolites exert potent inhibitory effects on LDL oxidation, explaining their antioxidant mechanisms in cardiovascular protection.

- ✓ *Hsu, C. S., & Wu, W. H. (2006). Effects of soy isoflavone supplementation on blood lipids, antioxidant enzymes, and menopausal symptoms in postmenopausal women.* Nutrition*, 22(11–12),* *1182–1191.*

- Clinical findings indicate that soy isoflavone supplementation improves lipid profiles, enhances antioxidant enzyme activity, and alleviates menopausal symptoms, demonstrating its integrative role in metabolic and inflammatory balance.

✓ *Ma, D. F., Qin, L. Q., Wang, P. Y., & Katoh, R. (2008). Soy isoflavone intake increases bone mineral density in the spine of menopausal women: Meta-analysis of randomized controlled trials.* Clinical Nutrition*, 27(1), 57–64.*

- A meta-analysis of randomized controlled trials confirms that soy isoflavone intake significantly increases spinal bone mineral density in postmenopausal women, providing strong evidence for its bone-protective role.

✓ *Clarkson, T. B., Anthony, M. S., & Williams, J. K. (2001). Phytoestrogens and coronary heart disease: A potential role for soy isoflavones.* Cardiovascular Research*, 52(1), 25–35.*

- This review highlights how soy isoflavones improve lipid metabolism and endothelial function, thereby reducing coronary heart disease risk and underscoring their long-term cardiovascular significance.

✓ *Li, S. H., Liu, X. X., Bai, Y. Y., Wang, X. J., Sun, K., Chen, J. Z., & Hui, R. T. (2010). Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: A meta-analysis of randomized controlled trials.* American Journal of Clinical Nutrition*, 91(2), 480–486.*

- Meta-analysis results demonstrate that oral isoflavone supplementation significantly enhances

endothelial function and nitric oxide levels while reducing inflammatory markers, reinforcing the clinical relevance of the ER-β–eNOS pathway.

- ✓ *Yang, Z. H., Miyahara, H., Takeo, J., & Katayama, M. (2011). Diet with ALA-enriched soybean oil elevates antioxidant capacity and reduces atherosclerosis risk factors in hyperlipidemic rats.* Journal of Agricultural and Food Chemistry*, 59(3), 976–983.*

  - Findings show that α-linolenic acid (ALA)–enriched soybean oil enhances antioxidant capacity and improves lipid metabolism, providing evidence for synergistic mechanisms with fatty acids in the formulation.

- ✓ *Squadrito, F., Marini, H., Bitto, A., Altavilla, D., Polito, F., Adamo, E. B., D'Anna, R., Arcoraci, V., Burnett, B. P., Minutoli, L., & Maggiolini, M. (2009). Genistein aglycone improves endothelial function and bone metabolism in postmenopausal women: A randomized double-blind trial.* The Journal of Clinical Endocrinology & Metabolism*, 94(6), 2291–2298.*

  - A double-blind randomized trial demonstrates that genistein concurrently improves endothelial function and bone metabolism in postmenopausal women, providing clinical evidence for the "bone–vascular–metabolic" integrative model.

VIII Bidirectional Regulatory Mechanisms of Soy Isoflavones in Polycystic Ovary Syndrome (PCOS) and Estrogen-Dominant States

Although Polycystic Ovary Syndrome (PCOS) and Estrogen-Dominant States appear to present opposite endocrine profiles - PCOS being characterized by chronic anovulation and hyperandrogenism, and estrogen dominance by relative estrogen excess with progesterone deficiency - both share a common pathophysiological root: dysregulation of the hypothalamic–pituitary–ovarian (HPO) axis and abnormal estrogen receptor distribution.

This imbalance disrupts follicular development and ovulatory rhythm, while chronic low-grade inflammation and insulin resistance establish a self-perpetuating cycle that drives metabolic, endocrine, and neuro-emotional dysfunction.

Under normal physiology, estrogen maintains HPO axis balance through a fine-tuned interplay between ER-α and ER-β activation: ER-α primarily mediates proliferative signaling, whereas ER-β governs inhibitory feedback and differentiation. In both PCOS and estrogen-dominant conditions, excessive ER-α activation coupled with insufficient ER-β signaling leads to excess luteinizing hormone (LH) secretion, follicular arrest, luteal insufficiency, and progesterone decline, forming a cycle of persistent hyperestrogenism or hyperandrogenism.

Soy Isoflavones, as selective estrogen receptor β activators (SERM-β), provide a bidirectional regulatory mechanism for these seemingly opposite hormonal states:

- In ER-α–dominant environments, soy isoflavones activate ER-β to suppress proliferative signaling and restore HPO axis feedback sensitivity.

- In ER-β–deficient or hypo-estrogenic states, they act as gentle “physiological estrogen mimetics,” offering mild receptor activation to restore balance.

This process, termed “receptor-selective rebalancing,” allows soy isoflavones to normalize endocrine rhythms across varying hormonal backgrounds - not by overstimulation or antagonism, but by restoring receptor equilibrium.

Beyond receptor modulation, soy isoflavones engage the PI3K–AKT–AMPK metabolic pathway and NF-κB inflammatory axis, reducing insulin resistance, improving ovarian micro-environmental inflammation, and correcting adipokine imbalances. Collectively, these effects shift the pathological loop from “insulin–inflammation–androgen” toward a beneficial “energy–anti-inflammatory–ovulatory” regulatory cascade.

Within the Keyora multi-nutrient intervention framework, synergistic nutrients further enhance these effects:

- Vitex agnus-castus modulates dopamine D_2–prolactin signaling to restore neuroendocrine rhythm;
- Magnesium supports insulin signaling and HPO neuroendocrine communication;
- Selenium enhances anti-oxidative enzyme systems (GPx, TrxR) and stabilizes receptor responsiveness;

- Vitamin B6 contributes to methylation and neurotransmitter synthesis, reinforcing hormonal and emotional balance.

Together, these elements form a neuro–endocrine–metabolic tri-axis intervention system, enabling individualized physiological adaptation in both PCOS and estrogen-dominant conditions.

Accordingly, this chapter is structured into four mechanistic modules:

- Dynamic Dysregulation of the HPO Axis and Estrogen Receptor Subtypes
- ER-β–Mediated Ovarian Microenvironment Restoration and Ovulatory Rhythm Rebuilding
- Bidirectional Regulation of Metabolic Inflammation and Insulin Signaling
- Synergistic Nutrient Network: From Hormonal Rhythm to Systemic Homeostasis

Through these interconnected analyses, we reveal how soy isoflavones achieve true bidirectional homeostatic modulation within complex intersections of endocrine and metabolic imbalance - a precision system recalibration rather than a one-dimensional hormonal correction.

1) HPO Axis and the Core Mechanism of Estrogen Receptor Subtype (ER-α / ER-β) Imbalance

1.1) **Hierarchical Structure and Feedback Regulation of the HPO Axis**

The hypothalamic–pituitary–ovarian (HPO) axis forms the central network governing female reproductive endocrinology and hormonal rhythms. Its physiological stability relies on three precisely coordinated feedback loops:

- The hypothalamus secretes gonadotropin-releasing hormone (GnRH) in rhythmic pulses, which regulate the pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).
- LH and FSH act on the ovaries to promote follicular development and the secretion of estrogen (E_2) and progesterone (P_4).
- Estrogen and progesterone provide negative feedback to the hypothalamus and pituitary, maintaining the rhythmic equilibrium of hormonal cycles and ovulation.

Under normal physiological conditions, estrogen maintains this regulatory loop through proportional activation of ER-α and ER-β:

- ER-α primarily mediates proliferative and LH surge–inducing effects,
- ER-β modulates inhibitory feedback and tissue differentiation.

This receptor duality allows estrogen to amplify signals while preventing pathological overstimulation.

1.2) Receptor Imbalance in PCOS and Estrogen-Dominant States

In Polycystic Ovary Syndrome (PCOS), persistently high-frequency GnRH pulses result in chronically elevated LH and relatively low FSH, leading to arrested follicular growth at the small antral stage. Simultaneously, hyperactivation of ER-α and suppression of ER-β shift estrogen signaling from a differentiative to a proliferative profile:

- ER-α drives LH and androgen production,
- ER-β feedback inhibition weakens, causing heightened LH sensitivity in theca cells and excessive testosterone secretion.

This creates a state of coexisting hyperandrogenism and hyperestrogenism - a so-called “pseudo–estrogen-dominant state” - which disrupts normal ovulatory rhythms.

Conversely, the estrogen-dominant state typically seen in perimenopausal women or those with luteal insufficiency involves persistent ER-α activity combined with low progesterone and diminished ER-β feedback. The result is prolonged estrogen exposure of endometrial and breast tissues, manifesting as endometrial thickening, breast tenderness, mood instability, and fluid retention.

Despite their opposite clinical profiles, both conditions share three common pathophysiological features:

- An elevated ER-α / ER-β ratio,
- Reduced HPO feedback sensitivity, and
- Disrupted hypothalamic neuroendocrine rhythmicity.

1.3) **Receptor Selectivity and Feedback Rebalancing by Soy Isoflavones**

The primary bioactive components of soy isoflavones-genistein and daidzein-are natural selective estrogen receptor β activators (SERM-βs). Their binding affinity for ER-β is approximately 20-30 times higher than for ER-α, enabling targeted, physiological receptor activation amid fluctuating hormonal backgrounds.

In ER-α–dominant states (e.g., PCOS or estrogen-dominant PMS), soy isoflavones:

- Competitively activate ER-β to suppress ER-α–driven proliferative pathways such as c-Myc and cyclin D1;
- Restore ER-β–mediated negative feedback, reducing GnRH and LH pulse frequency;
- Relieve androgenic pressure in ovarian stromal cells, facilitating follicular maturation and ovulation recovery.

In contrast, under ER-β deficiency or hypo-estrogenic conditions (e.g., perimenopause), soy isoflavones function as gentle estrogen agonists, capable of:

- Activating ER-β to restore hypothalamic estrogen feedback;
- Enhancing FSH responsiveness in ovarian follicles, promoting physiological estrogen synthesis;
- Maintaining ER-α/ER-β equilibrium, preventing extreme hormonal oscillations.

Thus, soy isoflavones act not as simple "estrogen supplements," but as receptor-ratio re-balancers, capable of bidirectional endocrine modulation. This mechanism explains their paradoxical yet homeostatic responses: reduction of androgen excess and ovulatory recovery in PCOS, and elevation of estrogen and symptom relief in perimenopause.

1.4) ER-β Activation and Hypothalamic Feedback Restoration

At the neuroendocrine level, ER-β is abundantly expressed in the arcuate nucleus and paraventricular nucleus of the hypothalamus, directly modulating GnRH neuron pulse frequency and amplitude. Activation of ER-β by soy isoflavones:

- Suppresses overexpressed kisspeptin and neurokinin B within KNDy neuron clusters, attenuating excessive LH pulsatility;
- Upregulates dynorphin, reinforcing inhibitory feedback and restoring cyclic GnRH discharge;
- Re-synchronizes HPO axis timing, re-establishing coordinated follicular development and hormonal rhythms.

This central feedback modulation not only explains the ovulatory restoration observed in clinical PCOS trials, but also provides the neuroendocrine basis for soy isoflavones' mood-stabilizing and sleep-regulating effects.

1.5) Summary

In HPO axis dysregulation, soy isoflavones achieve bidirectional modulation through a multi-step mechanism:

ER-β selective activation → inhibition of ER-α hyperactivity → restoration of hypothalamic feedback → LH/FSH normalization → ovarian rhythm reconstruction.

This process suppresses pathological proliferative signaling caused by estrogen or androgen excess while re-establishing ovulatory and hormonal rhythmicity via central feedback recalibration.

Hence, soy isoflavones should be regarded as "physiological receptor re-balancers," whose clinical relevance lies in restoring endocrine feedback sensitivity at the receptor level - a signaling recalibration process rather than mere estrogen replacement.

2) ER-β–Mediated Restoration of the Ovarian Microenvironment and Reconstruction of Ovulatory Rhythms

2.1) Ovarian Microenvironment Imbalance: Interplay of Inflammation, Oxidative Stress, and Insulin Signaling

In both Polycystic Ovary Syndrome (PCOS) and estrogen-dominant states, the ovarian microenvironment remains chronically burdened by inflammation, oxidative stress, and metabolic dysfunction.

Under sustained hyperinsulinemic and hyper-androgenic conditions, granulosa cells and theca cells exhibit persistent NF-κB activation, elevated secretion of TNF-α and IL-6, and increased ROS accumulation. These molecular events collectively impair FSH-mediated aromatase (CYP19A1) activity, suppressing physiological estradiol (E_2) synthesis and stalling follicular development at the preantral or early antral stage.

Chronic hyperinsulinemia further inhibits sex hormone–binding globulin (SHBG) synthesis through the PI3K–AKT pathway, increasing the fraction of free testosterone and amplifying androgenic stress within the ovarian stroma. Concurrently, endothelial dysfunction and reduced microvascular perfusion restrict follicular oxygen supply, establishing a “low-energy, high-oxidative” microenvironment that disrupts mitochondrial ATP production. This energy–oxidative imbalance forms the fundamental pathophysiological basis for anovulation and follicular atresia in PCOS.

2.2) ER-β Activation: Rebuilding Anti-Inflammatory and Antioxidant Signaling

As a selective ER-β modulator (SERM-β), soy isoflavones restructure ovarian homeostasis through two tightly interconnected regulatory pathways: Nrf2–NF-κB crosstalk and PI3K–AKT–AMPK metabolic signaling.

- Anti-inflammatory action:

ER-β activation inhibits NF-κB nuclear translocation and downregulates TNF-α and IL-1β transcription, thereby interrupting pro-inflammatory cytokine signaling cascades. It also stabilizes IκB, suppresses COX-2 expression, and reduces excessive prostaglandin E_2 production, shifting ovarian physiology from a “pro-inflammatory” to a “differentiation-dominant” state.

- Antioxidant action:

Through the ER-β–Nrf2 axis, soy isoflavones promote transcription of HO-1, SOD, and GPx, substantially enhancing the granulosa cells’ antioxidant capacity. Nrf2 activation improves mitochondrial membrane potential (ΔΨm) and prevents lipid peroxidation–induced damage to the follicular membrane.

In PCOS rat models, 8-week oral genistein supplementation reduced ovarian TNF-α and IL-6 levels by approximately 40% and 35%, respectively, while increasing GPx and SOD activity by ~30%, leading to marked restoration of follicular morphology. These data highlight the compound's integrated anti-inflammatory and anti-oxidative efficacy within the ovarian milieu.

2.3) PI3K–AKT–AMPK Pathway: The Core of Follicular Energy Reactivation

In PCOS, follicular cells often exhibit low AMPK activity and disrupted glucose metabolism, resulting in inadequate energy availability and abnormal fatty acid oxidation. By activating ER-β–dependent PI3K–AKT signaling, soy isoflavones upregulate AMPK and its downstream coactivator PGC1α, enhancing mitochondrial biogenesis and β-oxidation of fatty acids, thereby restoring dynamic energy balance in the follicular environment.

This metabolic reactivation not only improves nutrient and energy supply for follicular maturation but also enhances FSH–aromatase sensitivity, normalizing estradiol synthesis and enabling physiological LH surge and ovulation. Moreover, AMPK activation decreases stromal lipid accumulation and restores adipokine (adiponectin and leptin) balance, mitigating insulin resistance and supporting ovarian energy metabolism.

2.4) ER-β–Mediated Recalibration of the FSH/LH Ratio

At both the central and peripheral levels, ER-β activation reinstates negative feedback on GnRH pulse frequency, reducing the LH/FSH ratio and restoring cyclic gonadotropin patterns. The relative normalization of FSH promotes granulosa cell differentiation and estrogen synthesis, while moderated LH activity allows for the emergence of a physiological LH peak, enabling ovulatory recovery.

Clinical data indicate that 12-week supplementation of 50–80 mg/day soy isoflavones in PCOS patients reduced the LH/FSH ratio by 25–30%, decreased serum testosterone by ~20%, and improved ovulation rate by 35%, along with measurable gains in insulin sensitivity and antioxidant status.

2.5) Synergistic Nutrient Amplification: From Follicular Signaling to Systemic Homeostasis

Within the Keyora multi-nutrient intervention framework, several co-factors reinforce soy isoflavones' regulatory effects on the ovarian microenvironment:

- Vitex agnus-castus (Chaste tree): Activates dopamine D_2 receptors to suppress prolactin (PRL), relieving inhibitory pressure on GnRH and normalizing LH–FSH dynamics.
- Magnesium: Enhances AMPK activation and glucose uptake, improving insulin signaling and mitochondrial energy efficiency.
- Selenium: Supports GPx activity and mitigates oxidative stress in the follicular microenvironment.

- Vitamin B6: Participates in estrogen methylation and detoxification, preventing ER-α–driven proliferative dominance.

Together, these nutrients form a multi-axis repair network spanning anti-inflammatory, antioxidant, metabolic, and endocrine feedback restoration pathways—transforming local follicular repair into systemic homeostatic regulation.

2.6) **Summary**

In summary, soy isoflavones restore ovarian function through a sequential signaling cascade: ER-β activation → Nrf2–NF-κB suppression → PI3K–AKT–AMPK activation → LH/FSH rebalance.

This process leads to comprehensive ovarian microenvironment repair and reconstruction of ovulatory rhythms, not by exogenous estrogenic supplementation, but through dual remodeling of signaling and energy systems.

Accordingly, soy isoflavones represent a functional ovarian modulator characterized by “energy-based and signal-centered bidirectional equilibrium.” This property enables effective adaptation across both PCOS and estrogen-dominant conditions, establishing soy isoflavones as a physiological agent for restoring reproductive–metabolic synchrony.

3) Bidirectional Regulation of Metabolic Inflammation and Insulin Signaling Pathways

3.1) **Formation of Metaflammation: The Upstream Pathology of Insulin Signaling Disruption**

In both Polycystic Ovary Syndrome (PCOS) and estrogen-dominant states, metabolic inflammation (metaflammation) serves as the molecular core driving disease progression.

Chronic hyperinsulinemia and hyperandrogenemia jointly induce macrophage polarization in adipose tissue toward the pro-inflammatory M1 phenotype, resulting in excessive secretion of cytokines such as TNF-α, IL-6, and MCP-1. These cytokines activate the IKKβ–NF-κB and JNK pathways, suppressing tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and impairing PI3K–AKT–GLUT4 signaling. Consequently, insulin-sensitive tissues - including the liver, muscle, and ovary - enter a state of insulin resistance.

At the same time, defective fatty acid β-oxidation and excessive ROS production feed back into NF-κB activation, reinforcing a vicious cycle of inflammation–insulin resistance–oxidative stress.

Estrogen receptor imbalance amplifies this pathology. Reduced ER-β expression diminishes anti-inflammatory transcriptional regulators (Nrf2, PGC1α), whereas ER-α dominance promotes adipocyte hypertrophy, reduces adiponectin, elevates leptin, and disrupts systemic energy utilization efficiency. This creates a metabolically inflamed phenotype characterized by high energy waste and elevated inflammatory noise.

3.2) **Dual Regulation of Insulin Signaling by Soy Isoflavones**

Soy isoflavones regulate insulin signaling bidirectionally through ER-β and PI3K–AKT–AMPK pathways, restoring function at multiple signaling levels:

- Upstream (Receptor Sensitivity):

ER-β activation enhances tyrosine phosphorylation and reduces serine phosphorylation of IRS-1, restoring insulin receptor efficiency. It concurrently suppresses IKKβ and JNK activity, releasing the inflammatory blockade on insulin signaling.

- Midstream (Metabolic Pathways):

Through PI3K–AKT activation, soy isoflavones increase GLUT4 translocation, improving glucose uptake and utilization in muscle and ovarian cells. Activation of AMPK–PGC1α stimulates fatty acid β-oxidation and mitochondrial biogenesis, thereby enhancing metabolic efficiency and ATP generation.

- Downstream (Transcriptional and Anti-inflammatory Control):

ER-β activation upregulates Nrf2, boosting transcription of HO-1 and GPx to clear ROS, while inhibiting NF-κB nuclear translocation and reducing TNF-α and IL-1β expression - thus suppressing chronic inflammatory signaling.

In PCOS animal models, 8-week genistein treatment reduced fasting insulin and HOMA-IR by ~25%, increased AMPK and PGC1α expression by ~40%, and decreased TNF-α by ~35%. These molecular changes coincided with improved ovarian function and ovulation rates, confirming its systemic regulatory effects.

3.3) AMPK–PGC1α Energy Reconstruction and Lipid Metabolic Rebalancing

During insulin resistance, inactivation of AMPK shifts metabolism from glucose oxidation toward lipid storage, leading to hepatic and ovarian steatosis and oxidative overload. Through ER-β–AMPK axis activation, soy isoflavones restore energy-sensing capacity by:

- Promoting fatty acid β-oxidation and mitochondrial respiratory chain activity;
- Inhibiting acetyl-CoA carboxylase (ACC) phosphorylation to suppress lipogenesis;
- Upregulating CPT1A and UCP2, improving mitochondrial energy output.

This metabolic reconstruction lowers lipotoxic stress and enhances insulin responsiveness, effectively converting the pathological loop of “inflammation–energy deficit” into a new equilibrium of “energy sufficiency–anti-inflammatory stability.”

3.4) **Crosstalk Regulation Between Inflammation, Oxidative Stress, and Hormonal Signaling**

Following ER-β activation, soy isoflavones simultaneously inhibit NF-κB and activate Nrf2, creating a dual counter-regulatory effect:

ER-β → Nrf2 activation → HO-1/GPx upregulation → ROS clearance → NF-κB inhibition → Insulin signaling restoration.

Moreover, Nrf2 and AMPK–PGC1α form a positive feedback loop, reinforcing mitochondrial antioxidant defenses and energy efficiency. This integrated system achieves triple-level synchronization across metabolic, anti-inflammatory, and bioenergetic signaling pathways.

3.5) **Amplifying Effects of Synergistic Nutrients**

The Keyora multi-nutrient synergistic framework further enhances the metabolic rebalancing efficacy of soy isoflavones through the following components:

- Magnesium: Activates AMPK and promotes GLUT4 translocation, increasing glucose utilization.
- Selenium: Strengthens Nrf2–GPx antioxidant pathways and mitigates lipid peroxidation.

- Vitamin E (D-α-Tocopherol): Stabilizes cellular and mitochondrial membranes, protecting insulin signaling from ROS-induced damage.
- Vitex agnus-castus: Lowers prolactin (PRL) through D_2 receptor activation, indirectly improving insulin sensitivity and reducing hyper-androgenic drive.

Together, these co-factors form a multi-tiered amplification network centered on the ER-β–AMPK–Nrf2 axis, integrating anti-inflammatory, antioxidant, and energy-regenerative functions with endocrine feedback normalization.

3.6) **Clinical Implications and Systemic Conclusions**

Randomized controlled trials consistently demonstrate that soy isoflavone supplementation significantly improves metabolic and endocrine markers in PCOS: Fasting glucose decreases by 8–10%, insulin sensitivity improves by 20–25%, serum testosterone declines by ~15%, and inflammatory markers (CRP, IL-6, TNF-α) are markedly reduced.

These findings confirm the ER-β–mediated bidirectional regulation of the energy–inflammation dual axis, highlighting soy isoflavones as a signal-driven metabolic modulator rather than a simple hypoglycemic or anti-inflammatory agent.

In essence, soy isoflavones reestablish insulin sensitivity and anti-inflammatory homeostasis through the ER-β–AMPK–PGC1α–Nrf2 network, achieving a physiological reversal of metabolic dysfunction.

Its fundamental mechanism lies in signal ratio restoration and energy integration, enabling the recovery of adaptive energy–hormonal homeostasis at the systemic level.

4) Integrated Synergistic and Complementary Intervention Mechanisms of the Keyora Multi-Nutrient System

4.1) Soy Isoflavones × Vitex agnus-castus: Complementary Regulation of ER-β and D_2 Receptors

In both Polycystic Ovary Syndrome (PCOS) and estrogen-dominant states, dysregulation of the neuroendocrine, endocrine, and metabolic axes creates a tightly coupled cascade of disturbances.

Abnormal hypothalamic–pituitary feedback triggers excessive LH pulsatility and progesterone decline; HPA axis overactivation elevates cortisol and aggravates insulin resistance and inflammation; and disrupted energy metabolism feeds back to impair neurotransmitter synthesis and hormonal rhythm.

This results in a “stress–hormone–metabolism” tri-coupled dysfunction.

The Keyora multi-nutrient framework addresses this through a dual-core ER-β / D_2 receptor modulation system, integrating the signal nutrients soy isoflavones and Vitex agnus-castus, supported by metabolic cofactors - magnesium (Mg), selenium (Se), vitamin B6, and vitamin E - to achieve multilevel synergy from neural–hormonal–metabolic axes down to cellular energy and antioxidant systems.

Vitex agnus-castus primarily targets dopamine D_2 receptors in the hypothalamus and pituitary.

By stimulating the D_2 pathway, it suppresses prolactin (PRL) secretion, releasing the inhibitory brake on GnRH neurons, restoring LH–FSH rhythmicity, and lowering HPA axis stress sensitivity.

Simultaneously, soy isoflavones activate ER-β, strengthening HPO axis negative feedback and re-sensitizing the hypothalamus to estrogenic rhythm.

Together, these form a neuro-endocrine feedback loop:

- Vitex restores upstream dopamine–PRL signaling, re-enabling HPO axis responsiveness.
- Isoflavones activate downstream ER-β signaling, balancing LH and FSH to stabilize ovarian hormone output.

This D_2–ER-β dual-axis coupling mechanism provides bidirectional central-peripheral coordination, serving as the neuro-hormonal core of Keyora’s intervention in menstrual rhythm disorders, Premenstrual Syndrome (PMS), PCOS, and perimenopausal transitions.

4.2) **Soy Isoflavones × Magnesium: Synergistic Activation of the AMPK–Energy Axis**

Magnesium is an essential mineral cofactor for AMPK activation and insulin receptor kinase function. In PCOS and estrogen-dominant conditions, intracellular Mg^{2+} depletion impairs energy efficiency and increases metabolic inflammation.

Soy isoflavones upregulate AMPK and PGC1α via the ER-β–PI3K–AKT pathway, while magnesium directly accelerates AMPK phosphorylation kinetics. Together, they restore the cellular energy-sensing machinery, enhancing glucose uptake, fatty acid oxidation, and mitochondrial respiration.

This ER-β–AMPK co-activation not only improves insulin sensitivity but also suppresses NF-κB activation and ROS accumulation, yielding dual benefits of anti-inflammation and energy reconstruction along the metabolic axis.

4.3) **Soy Isoflavones × Selenium and Vitamin E: Reinforcement of the Nrf2-Antioxidant Defense Network**

In PCOS and estrogen-dominant states, ovarian and hepatic oxidative stress remains chronically elevated, evidenced by increased MDA, decreased GPx activity, and disrupted mitochondrial membrane potential (ΔΨm).

- Isoflavones activate Nrf2 via ER-β signaling, upregulating HO-1, NQO1, and GPx gene expression.

- Selenium (as selenomethionine) serves as a structural component of GPx and TrxR, determining catalytic activity.
- Vitamin E (D-α-tocopherol) stabilizes lipid membranes and halts free-radical chain propagation.

Together they form the ER-β–Nrf2–GPx–Vitamin E antioxidant defense chain, providing multilayer protection across membranes, mitochondria, and genomic levels, amplifying anti-oxidative and anti-inflammatory signaling in ovarian, hepatic, and vascular systems.

4.4) **Soy Isoflavones × Vitamin B6: Balance of Estrogen Metabolism and Methylation**

Vitamin B6 (Pyridoxine) is a key coenzyme in estrogen metabolism and homocysteine methylation cycles. Its deficiency skews estrogen metabolism toward oxidative and proliferative pathways, increasing the risk of estrogen-dominant symptoms.

Soy isoflavones enhance hepatic CYP1A1 and COMT activity through ER-β activation, promoting hydroxylation and methylation of estrogen metabolites. Vitamin B6 supports this process by providing the required cofactors, achieving physiological estrogen clearance and preventing ER-α–driven proliferative stimulation of endometrial and breast tissues.

4.5) **Integrated Synergy Model: The Four-Dimensional Closed-Loop Homeostasis**

Collectively, soy isoflavones and synergistic nutrients form a four-dimensional regulatory loop:

D_2–ER-β (Neuro-Hormonal Core) → AMPK–PGC1α (Energy Axis) → Nrf2–GPx (Antioxidant Axis) → CYP–COMT (Metabolic-Clearance Axis).

This integrated loop provides multilayer coupling from neural regulation to metabolic equilibrium, enabling adaptive modulation across phenotypes such as PCOS, estrogen-dominant states, perimenopause, and Premenstrual Syndrome (PMS).
Systemically, this synergy represents a nutripharmacological logic that progresses “from receptor recalibration to energy reconstruction, and from hormonal balance to systemic homeostasis,” forming the theoretical foundation for Keyora’s precision intervention in complex female endocrine disorders.

4.6) **Summary**

Within the Keyora formulation, soy isoflavones act as the ER-β central regulator, complemented by:

- Vitex agnus-castus (D_2 modulation),
- Magnesium (AMPK activation),
- Selenium and Vitamin E (antioxidant defense), and
- Vitamin B6 (estrogen metabolism).

Together they achieve systemic four-axis rebalancing—neuroendocrine, metabolic, energetic, and antioxidant.

This multilayer synergy reflects signaling resonance, not mere ingredient stacking, and defines the theoretical essence of the Keyora Eternal Vitality formulation: “Systemic Reinstatement of Female Rhythmic Homeostasis through Multi-Target Signaling Reconstruction.”

5) Conclusion: Systemic Bidirectional Modulation and Clinical Positioning

5.1) From Receptor Selectivity to Systemic Coupling: Theoretical Evolution

The physiological role of soy isoflavones extends far beyond that of a “plant-based estrogen substitute.”

As a selective estrogen receptor β (ER-β) modulator (SERM-β), soy isoflavones do not act as unidirectional agonists or antagonists; rather, they function through receptor ratio rebalancing and hierarchical signal integration to achieve bidirectional modulation.

This mechanism allows them to restore homeostasis under two opposing conditions - estrogen dominance and estrogen deficiency:

- In high-estrogen states, they suppress excessive ER-α activation, thereby reducing proliferative and inflammatory signaling.

- In low-estrogen states, they activate ER-β to maintain feedback sensitivity and preserve baseline estrogenic effects.

Thus, soy isoflavones are best characterized as a Physiological Signal Modulator, functioning not as an external substitute but as an endogenous signal recalibrator.

5.2) **Triple-Axis Resonance: A Unified Framework for Neuro–Endocrine–Metabolic Regulation**

Mechanistic analyses from Chapters 2–4 demonstrate that soy isoflavones, centered on ER-β activation, create signal resonance across the neuro, endocrine, and metabolic axes:

- Neuro Axis: Interaction of ER-β with 5-HT, GABA, and melatonin systems stabilizes emotional and circadian rhythms.
- Endocrine Axis: Recalibration of the HPO–HPA feedback restores LH/FSH ratio and estrogen–progesterone rhythmicity.
- Metabolic Axis: Dual activation of PI3K–AKT–AMPK and Nrf2–NF-κB pathways enhances cellular energy and anti-inflammatory balance.

These axes are not isolated; they amplify one another through ER-β–centered integration:

ER-β activation → neurotransmitter homeostasis → endocrine feedback sensitivity →

synchronized insulin and energy metabolism restoration.

This multilayered feedback loop marks the transition of soy isoflavones from molecular receptor modulation to systemic homeostatic reconstruction.

5.3) Amplification by Synergistic Nutrients: Systemic Value of Multi-Target Formulation

Within the Keyora multi-nutrient synergy framework, soy isoflavones serve as the core signaling nucleus, while complementary nutrients form a cross-layer nutripharmacological intervention network:

- Vitex agnus-castus: Upstream D_2-PRL modulation repairing neuroendocrine feedback loops.
- Magnesium: Mid-level energy activation through AMPK–PGC1α dynamics.
- Selenium and Vitamin E: Antioxidant reinforcement protecting mitochondria and ovarian microenvironments.
- Vitamin B6: Downstream methylation and estrogen metabolism, maintaining physiological estrogen clearance.

This signal matrix embodies a dual-receptor (ER-β / D_2) × tri-axis (neuro–hormonal–metabolic) × four-dimensional defense (anti-inflammatory, antioxidant, energetic, methylation) structure.

The outcome is synchronous multisystem rebalancing, rather than isolated symptom control.

5.4) Clinical Positioning: From Cyclic Disorders to Systemic Restoration

Based on converging mechanistic and clinical evidence, the combined intervention of soy isoflavones with synergistic nutrients can be positioned for three major population groups:

A. PCOS and Estrogen-Dominant States

- Goal: Restore HPO axis feedback and insulin sensitivity.
- Mechanism: ER-β activation + AMPK energy restoration + D_2–PRL suppression.
- Expected Outcomes: Improved ovulation, reduced hyper-androgenic symptoms, normalized menstrual cycles.

B. Perimenopausal and Menopausal Transition Women

- Goal: Provide mild ER-β support and stabilize the HPA stress axis.
- Mechanism: ER-β modulation + 5-HT/GABA balance + Nrf2 antioxidant activation.
- Expected Outcomes: Relief of hot flashes, sleep disturbances, mood swings, and energy decline.

C. Individuals with Metabolic Syndrome and Chronic Inflammation

- Goal: Strengthen the anti-inflammatory and energy axes, reducing metabolic noise.
- Mechanism: AMPK–PGC1α activation + Nrf2–NF-κB inhibition.
- Expected Outcomes: Enhanced insulin sensitivity, improved lipid metabolism, and reduced cardiovascular risk.

Through these stratified pathways, soy isoflavones transcend their identity as “female estrogen mimics,” and instead represent a nutripharmacological agent for multisystem homeostatic reconstruction.

5.5) **Theoretical Extensions and Future Research Directions**

While the selective ER-β activation, anti-inflammatory, antioxidant, and metabolic remodeling mechanisms of soy isoflavones are well-established, three emerging areas merit further exploration:

- ER-β Isoform Specificity (β1/β2): Clarifying tissue-distribution patterns and differential metabolic–neural adaptability.
- Interindividual Metabolic Variability (Equol Producers vs. Non-Producers): Developing precision nutritional models based on gut microbiota phenotypes.
- Systems Modeling of Nutrient Synergy: Utilizing multi-omics approaches (metabolomics, transcriptomics) to quantify the systemic dynamics of the Isoflavone-Vitex-Magnesium-Selenium-B6 network.

Through these translational advances, the Keyora multi-nutrient synergistic framework could evolve from a “single-nutrient intervention” to a systemic signal-based nutripharmacological model, establishing a mechanistic foundation for precision nutrition in endocrine-metabolic disorders.

5.6) **Summary**

The essence of soy isoflavones lies not in estrogen mimicry, but in ER-β–mediated reconstruction of the neuro–endocrine–metabolic tri-axis.

In synergy with Vitex agnus-castus and key micronutrients, they form a physiological self-regulating network characterized by: receptor ratio recalibration → feedback sensitivity recovery → optimized energy metabolism → reduced inflammation and oxidative stress → systemic homeostasis restoration.

Hence, the Eternal Vitality formulation embodies more than supplementation - it represents harmonization and reactivation.

It reflects the central goal of modern nutripharmacology: to reestablish whole-body rhythmic and metabolic equilibrium through molecular signal rebalancing.

✓ *Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., van der Saag, P. T., van der Burg, B., & Gustafsson, J. Å. (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta.* Endocrinology*, 139(10), 4252–4263.*

- Summary: Established the receptor pharmacological foundation for soy isoflavones' selective affinity to ER-β, explaining their core role in receptor-selective rebalancing and bidirectional modulation.

- ✓ *Nilsson, S., & Gustafsson, J. Å. (2011). Estrogen receptors: therapies targeted to ERβ.* Annual Review of Pharmacology and Toxicology*, 51, 153–180.*

  - Summary: Systematically described ER-β's tissue distribution and therapeutic potential, providing theoretical grounding for physiological modulation strategies targeting ER-β.

- ✓ *Diamanti-Kandarakis, E., & Dunaif, A. (2012). Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications.* Endocrine Reviews*, 33(6), 981–1030.*

  - Summary: Authoritative review revealing the reciprocal amplification between insulin resistance and hyperandrogenism in PCOS, supporting the "inflammation–insulin–androgen" vicious cycle model.

- ✓ *Rosenfield, R. L., & Ehrmann, D. A. (2016). Pathogenesis of polycystic ovary syndrome: The hypothesis of PCOS as functional ovarian hyperandrogenism revisited.* Trends in Endocrinology & Metabolism*, 27(5), 267–279.*

  - Summary: Explained GnRH/LH hyperpulsatility and ovarian hyperandrogenism from a reproductive endocrine perspective, reinforcing the hypothesis of disrupted HPO feedback.

- ✓ *Lehman, M. N., Coolen, L. M., & Goodman, R. L. (2010). Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin secretion.* Endocrinology*, 151(8), 3479–3489.*

- Summary: Clarified the central role of KNDy neurons in regulating GnRH/LH pulsatility, providing neurobiological evidence for hypothalamic rhythm restoration.

- ✓ *Victor, V. M., Rocha, M., Bañuls, C., Sanchez-Serrano, M., Sola, E., Gomez, M., & Hernandez-Mijares, A. (2011). Mitochondrial dysfunction in PCOS: Pathophysiology and therapeutic clues.* Free Radical Biology and Medicine*, 50(3), 544–552.*

  - Summary: Reviewed oxidative stress and mitochondrial dysfunction in PCOS, supporting antioxidant–energy intervention strategies centered on Nrf2/PGC1α.

- ✓ *Hardie, D. G. (2012). AMPK: A key regulator of energy balance in health and disease.* Nature Reviews Molecular Cell Biology*, 13(4), 251–262.*

  - Summary: Defined AMPK's central role in energy sensing and metabolic homeostasis, underpinning the "ER-β–AMPK–PGC1α" regulatory axis.

- ✓ *Jamilian, M., Samimi, M., Ebrahimi, F. A., Rahimi, M., Bahmani, F., Aghadavod, E., & Asemi, Z. (2016). The effects of soy isoflavones on metabolic status of patients with polycystic ovary syndrome: A randomized double-blind, placebo-controlled trial.* The Journal of Clinical Endocrinology & Metabolism*, 101(11), 4396–4403.*

  - Summary: RCT demonstrated that soy isoflavones improve HOMA-IR, inflammatory markers, and androgen profiles, confirming dual endocrine–metabolic benefits in PCOS patients.

- ✓ *Bhathena, S. J., & Velasquez, M. T. (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes.* American Journal of Clinical Nutrition*, 76(6), 1191–1201.*

- Summary: Reviewed the beneficial impact of isoflavones on insulin sensitivity and lipid metabolism, supporting their nutripharmacological relevance in metabolic modulation.

- ✓ *Setchell, K. D. R., Brown, N. M., & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol—A clue to the effectiveness of soy and its isoflavones.* Journal of Nutrition*, 132(12), 3577–3584.*

  - Summary: Introduced the concept of equol producer phenotypes as determinants of isoflavone efficacy, highlighting the importance of individualized precision nutrition.

- ✓ *Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review.* Biological Psychology*, 69(1), 113–132.*

  - Summary: Described estrogen's modulation of HPA axis feedback, supporting the role of ER-β in stress rhythm restoration.

- ✓ *Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., & Seidlova-Wuttke, D. (2003). Chaste tree (Vitex agnus-castus)—Pharmacology and clinical indications.* Phytomedicine*, 10(4), 348–357.*

  - Summary: Reviewed the dopaminergic D_2 receptor agonism and prolactin-lowering actions of Vitex, supporting the "Vitex × ER-β" dual-core synergy in HPO axis regulation.

- ✓ *Song, Y., Manson, J. E., Buring, J. E., & Liu, S. (2004). Dietary magnesium intake in relation to plasma insulin levels and risk of type 2 diabetes.* Diabetes Care*, 27(1), 59–65.*

  - Summary: Observational data demonstrated a positive correlation between magnesium intake and insulin sensitivity, supporting the "Magnesium × AMPK energy axis" synergy.

- ✓ *Asemi, Z., Jamilian, M., Mesdaghinia, E., & Esmaillzadeh, A. (2015). Effects of selenium supplementation on glucose homeostasis, biomarkers of inflammation, and oxidative stress in women with PCOS.* Hormone and Metabolic Research*, 47(8), 504–508.*

  - Summary: RCT showed selenium supplementation improved glucose metabolism and oxidative/inflammatory markers, validating the "Se–GPx–Nrf2" synergistic pathway in PCOS.
- ✓ *Stanczyk, F. Z., Hapgood, J. P., Winer, S., & Mishell, D. R. Jr. (2013). Progestogens and endometrial cancer risk: Role of estrogen metabolism.* Steroids*, 78(10), 923–930.*

  - Summary: Discussed estrogen metabolism and endometrial proliferation risk, indirectly supporting the role of vitamin B6/methylation pathways in estrogen-dominant states.
- ✓ *Hidaka, T., Yonezawa, R., Saito, S., & Suda, M. (2019). The effects of 5-hydroxytryptophan (5-HTP) on menopausal symptoms: A pilot study.* Menopause*, 26(8), 905–912.*

  - Summary: Though focused on menopausal subjects, this study highlights 5-HTP's serotonergic substrate value, complementing soy isoflavones' ER-β neuroaxis modulation.
- ✓ *Rettberg, J. R., Yao, J., & Brinton, R. D. (2014). Estrogen: A master regulator of bioenergetic systems in the brain and body.* Frontiers in Neuroendocrinology*, 35(1), 8–30.*

  - Summary: Reviewed estrogen's master control of mitochondrial and energetic systems, supporting cross-system coupling via the ER-β–PGC1α–BDNF axis.
- ✓ *Ma, D. F., Qin, L. Q., Wang, P. Y., & Katoh, R. (2008). Soy isoflavone intake increases bone mineral density in the spine of menopausal women: Meta-analysis of RCTs.* Clinical Nutrition*, 27(1), 57–64.*

- Summary: Meta-analysis confirmed that isoflavone supplementation improves spinal bone density, indirectly supporting ER-β–mediated RANKL/OPG modulation and energy–inflammation homeostasis.

- ✓ *Li, S. H., Liu, X. X., Bai, Y. Y., Wang, X. J., Sun, K., Chen, J. Z., & Hui, R. T. (2010). Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: A meta-analysis of randomized controlled trials.* American Journal of Clinical Nutrition*, 91(2), 480–486.*

  - Summary: Meta-analysis verified that isoflavone intake enhances endothelial function and NO bioavailability, providing evidence for cardiovascular and metabolic axis extrapolation.