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## The mechanism of action of indole-3-propionic acid on bone metabolism

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Indole-3-propionic acid (IPA), a metabolite produced by gut microbiota through tryptophan metabolism, has recently been identified as playing a pivotal role in bone metabolism. IPA promotes osteoblast differentiation by upregulating mitochondrial transcription factor A (Tfam), contributing to increased bone density and supporting bone repair. Simultaneously, it inhibits the formation and activity of osteoclasts, reducing bone resorption, possibly through modulation of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway and downregulation of osteoclast-associated factors, thereby maintaining bone structural integrity. Additionally, IPA provides indirect protection to bone health by regulating host immune responses and inflammation *via* activation of receptors such as the Aryl hydrocarbon Receptor (AhR) and the Pregnane X Receptor (PXR). This review summarizes the roles and signaling pathways of IPA in bone metabolism and its impact on various bone metabolic disorders. Furthermore, we discuss the therapeutic potential and limitations of IPA in treating bone metabolic diseases, aiming to offer novel strategies for clinical management.

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### Introduction

In the last twenty years, there has been a growing fascination with the interplay between gut microbiota and bone metabolism.<sup>1</sup> As a result, the field of research known as “osteomicrobiology” was coined.<sup>2</sup> The complex network between gut microbiota and bones is implicated in the pathological progression of various bone disorders linked to imbalances in bone metabolism.<sup>1</sup> Disruptions in the gut microbiota are associated with bone deterioration under certain pathophysiological conditions,<sup>3</sup> including estrogen deficiency,<sup>4</sup> aging,<sup>5</sup> obesity<sup>6</sup> and long-term glucocorticoid use.<sup>7</sup> Extensive research indicates that the gut microbiota can not only stimulate bone loss by activating osteoclasts responsible for bone resorption but also regulate osteoblasts involved in bone formation, thereby providing anabolic stimulation to the bone.<sup>8–15</sup> These findings have intensified interest in exploring the mechanisms by which the gut microbiota may influence the regulation of bone metabolism.

Recent studies on fecal metabolomics have revealed a communication channel between bone metabolism and the metabolism of tryptophan (Trp).<sup>16</sup> Indole-3-propionic acid (IPA), a metabolite of Trp, plays a role in the regulation of bone

metabolism.<sup>17,18</sup> Research indicates that IPA can influence the immune system *via* signaling pathways such as the Aryl hydrocarbon Receptor (AhR) and the Pregnane X Receptor (PXR), reducing inflammatory responses in bone tissue, thereby lowering osteoclast activity and preserving bone mass.<sup>19</sup> Additionally, IPA shows significant effects in modulating oxidative stress, helping to alleviate oxidative damage associated with osteoporosis, which may be beneficial for bone health.<sup>20,21</sup> Based on this, understanding the impact of IPA on bone metabolism is vital for the development of targeted medications for bone disorders. This review will summarize the current insights into the effects of IPA on bone metabolism and explore its potential clinical significance in treating bone metabolic ailments.

### The function and signaling pathway of IPA

The diversity and stability of the gut microbiota play essential roles in host health and nutrient metabolism.<sup>22</sup> Increasing evidence indicates that metabolites produced by the gut microbiota serve as crucial mediators in the interplay between dietary intake and host health.<sup>23</sup> Tryptophan, an essential amino acid, is primarily obtained from dietary sources, especially protein-rich foods like meat, milk, eggs, and chocolate.<sup>24</sup> Most dietary proteins are digested and absorbed in the upper gastrointestinal tract through proteolysis. However, depending on intake levels, some proteins, peptides, and

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amino acids reach the colon through peristalsis.<sup>25</sup> In the colon, these amino acids are transformed by the microbiota *via* deamination or decarboxylation, resulting in a variety of small-molecule metabolites.<sup>26</sup> IPA, a metabolite of dietary tryptophan produced by the gut microbiota that was first discovered in 1923,<sup>17,27</sup> is primarily produced by *C. sporogenes*, *Peptostreptococcus anaerobius* CC14N, and three strains of *Clostridium cadaveris*.<sup>28</sup> The presence of the *fldBC* gene cluster serves as a reliable indicator of IPA production.<sup>28</sup> Trp undergoes metabolism primarily through the serotonin,<sup>29</sup> canine uric acid,<sup>30</sup> and intestinal microbial pathways.<sup>31</sup> Nevertheless, only microbial metabolism can generate IPA.<sup>32</sup> Trp not absorbed from the diet is taken up by symbiotic microbiota, with gut microbiota like *Clostridium*, *Bacteroides*, and *Bifidobacterium* absorbing this amino acid. These bacteria produce various enzymes leading to the formation of metabolites such as Indole-3-acetamide, Tryptamine, Indole-3-pyruvate acid (IPyA), and Indole.<sup>28,33</sup> Trp is transformed into IPyA with the participation of aromatic amino acid aminotransferase, then converted to indolelactic acid (ILA) through an oxidation–reduction reaction involving phenylalanine dehydrogenase. Subsequently, bacteria species containing the benzoyl-CoA ligase and its activator benzoyl-CoA undergo dehydration to convert ILA to indoleacrylic acid (IA). IA is further transformed into IPA through acyl-CoA dehydrogenase with the assistance of acyl-CoA ligase.<sup>28,30,34</sup> Extensive research shows that variations in gut microbial composition and dietary patterns can significantly influence IPA production.<sup>35–39</sup> Circulating IPA levels are associated with fiber intake,<sup>35,37</sup> with both the alpha and beta diversity of the gut microbiome positively correlated with IPA levels.<sup>36,39</sup> Furthermore, different dietary structures can affect IPA concentrations: a Mediterranean diet is linked to increased IPA levels, while consuming fried meats reduces gut microbiome richness, leading to lower IPA concentrations.<sup>40,41</sup> Studies on fermented dairy products in human serum metabolomics revealed that postprandial IPA and indole acetic acid levels were lower in the yogurt group compared to the milk group.<sup>42</sup> Additionally, plasma IPA levels in mice on a standard diet were significantly higher than those on a ketogenic diet.<sup>43</sup> In summary, dietary patterns can shape the gut microbiome's composition, potentially resulting in individual differences in IPA production.

IPA acts as a mediator for biological activities by interacting with the AhR and PXR.<sup>33</sup> AhR and PXR are widely distributed in various tissues and can induce the expression of downstream genes, playing essential roles in processes such as inflammation, cell proliferation and apoptosis.<sup>44,45</sup> The nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway is the canonical signaling pathway of IPA and is typically present in many cellular responses. When IPA enters the ligand-binding domain of AhR and PXR, it binds to them, blocks the phosphorylation of inhibitor of nuclear factor kappa B kinase subunit beta (IKK $\beta$ ) and NF-kappaB inhibitor alpha (IkB $\alpha$ ),<sup>46,47</sup> thereby preventing the activation of the classical NF- $\kappa$ B signaling pathway.<sup>46</sup> Apart from NF- $\kappa$ B signaling pathway, IPA also plays a crucial role in subsequent biological processes through the Transforming

Growth Factor- $\beta$  (TGF- $\beta$ ) pathway and the phosphatidylinositol 3-kinase/serine-threonine kinase (PI3K/AKT) pathway.<sup>48,49</sup>

Recent research has increasingly highlighted the involvement of IPA in the pathophysiological processes of various diseases. Dragicevic *et al.* demonstrated that IPA could reverse mitochondrial dysfunction associated with Alzheimer's disease, offering effective protection to nerve cells against oxidative damage induced by amyloid beta (A $\beta$ ).<sup>50,51</sup> Moreover, IPA has been found to safeguard neurons from ischemia-induced damage by mitigating oxidative stress, lipid peroxidation, and oxidative DNA damage.<sup>52</sup> As an inflammatory related factor, IPA has shown promise in attenuating hepatic steatosis in high-fat diet (HFD) rats by inhibiting NF- $\kappa$ B signaling activation and reducing the production of proinflammatory cytokines.<sup>21</sup> Furthermore, IPA activates the AhR and enhances barrier function by upregulating the expression of claudins and other tight junction proteins,<sup>21,49,53</sup> while simultaneously downregulating the expression of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin(IL)-1 $\beta$ , and IL-6,<sup>21</sup> thereby mitigating intestinal inflammatory responses. Additionally, IPA has been associated with obesity,<sup>54</sup> hypertension,<sup>55</sup> tuberculosis,<sup>56</sup> osteoarthritis,<sup>46</sup> and sarcopenia.<sup>57</sup>

## The effects of IPA on the bone metabolism

### Bone metabolism

The balance of bone metabolism is maintained through the homeostatic equilibrium between bone-forming osteoblasts and bone-resorbing osteoclasts. Osteoblasts synthesize osteoid molecules and release matrix vesicles to facilitate mineralization and bone formation, while osteoclasts secrete organic acids and proteases to breakdown and absorb bone matrix.<sup>58</sup>

Osteoclasts are large multinucleated cells, formed from the fusion of mononuclear progenitors of the monocyte/macrophage, originated from hematopoietic stem cell precursors (HSCs), which are responsible for bone resorption. The differentiation of osteoclasts is primarily regulated by macrophage colony-stimulating(M-CSF), receptor activator of NF- $\kappa$ B ligand (RANKL), and osteoprotegerin (OPG).<sup>58</sup> M-CSF binds to its receptor c-fms on early osteoclast precursors, promoting their proliferation and survival, thereby triggering the activation of the mitogen-activated protein kinases (MAPK) and PI3K. These signals ultimately inhibit the apoptosis of osteoclast precursors, allowing for their proliferation.<sup>59</sup> M-CSF also induces pre-osteoclasts to express the receptor activator of NF- $\kappa$ B (RANK) receptors, which bind to RANKL and initiate intracellular signals for osteoclast formation. RANKL is another main osteoclastogenic factor, which is a member of the TNF superfamily and is highly expressed in osteoblasts, osteocytes, lymph nodes and activated T lymphocytes.<sup>60–63</sup> When RANKL binds to RANK on the surface of osteoclasts and osteoclast precursors, it recruits TNF receptor-associated factor 6 (TRAF6) to the RANK intracellular domain, and activates a series of tran-

scription factors necessary for osteoclast differentiation, including NF- $\kappa$ B p50, p52, c-Fos, and nuclear factor of activated T cells (NFATc1).<sup>64</sup> NFATc1 acts as the master transcription factor that regulates osteoclast differentiation.<sup>65</sup> Upon stimulation by RANKL, NF- $\kappa$ B is shortly recruited to the NFATc1 promoter.<sup>66</sup> Subsequently, the overexpression of NFATc1 further induces osteoclast differentiation.<sup>67</sup> OPG acts as a soluble decoy receptor of RANKL, competing with RANK for binding to RANKL and activating the wingless-related integration site-beta-catenin (Wnt/ $\beta$ -catenin) signaling pathway. This results in the inhibition of RANKL-induced osteoclastogenesis and bone resorption.<sup>68</sup>

Osteoblasts, derived from mesenchymal stem cells (MSCs), are responsible for bone formation and matrix maturation.<sup>69</sup> Increased osteoblast activity promotes mineralization, facilitating new bone formation and remodeling.<sup>70</sup> The Wnt and bone morphogenetic proteins (BMPs) pathways stimulate osteoblast differentiation from MSCs by mediating osteoblast transcription factors.<sup>71</sup> Various transcription factors regulate osteogenic differentiation, with runt-related transcription factor 2 (Runx2) and osterix (Osx) considered as the master transcription factor involved in initiating and promoting osteogenic differentiation of MSCs.<sup>72,73</sup> Runx2, a member of the Runx transcription factor family, is crucial for the mesenchymal cells differentiation into osteoblasts by inhibiting differentiation into adipocytes and chondrocytes.<sup>74</sup> Runx2 upregulates osteoblast-related genes such as collagen type I alpha 1 chain (Col1a1), Alkaline phosphatase (ALP), bone gamma-carboxyglutamate protein (Bglap), bone sialoprotein (BSP) and osteocalcin (OCN). Osx, a member of the SP transcription factor family and downstream gene of Runx2,<sup>75</sup> downregulates the expression of type X collagen (Col X) and attenuates ALP enzyme activity, leading to chondrogenic gene activation and chondrocyte differentiation.<sup>76</sup>

The coupling of bone formation and resorption involves the interaction of a wide range of cell types at different stages of differentiation and secretion of related factors. This includes growth factors released from bone resorption, soluble and membrane products of osteoclasts and their precursors, as well as signals from osteocytes. During bone remodeling, osteoclasts and osteoblasts communicate through EphrinB2-EphB4, FAS Ligand-FAS, and semaphorin 3A-neuropilin-1, which can suppress osteoclast differentiation and promote osteoblasts differentiation.<sup>77</sup> TGF- $\beta$  and Insulin Growth Factor-1 (IGF-1) released from bone absorption promote osteoblast differentiation by recruiting of MSCs.<sup>77</sup> Additionally, factors like platelet-derived growth factor-BB, sphingosin-1-phosphate (S1P), and BMPs act as coupling factors to further promote osteoblast differentiation.<sup>78</sup> On the other hand, osteoblasts secrete M-CSF, RANKL and Wnt gene family 5A (WNT5A) to promote osteoclast differentiation.<sup>62,79,80</sup>

Inflammatory cytokines, such as TNF- $\alpha$  and IL-1, are crucial in coordinating the balance between bone resorption and formation. TNF- $\alpha$  can attract osteoclasts and, in synergy with IL-1, upregulate the expression of RANKL, promoting osteoclast differentiation.<sup>81</sup> Conversely, anti-inflammatory cytokines like

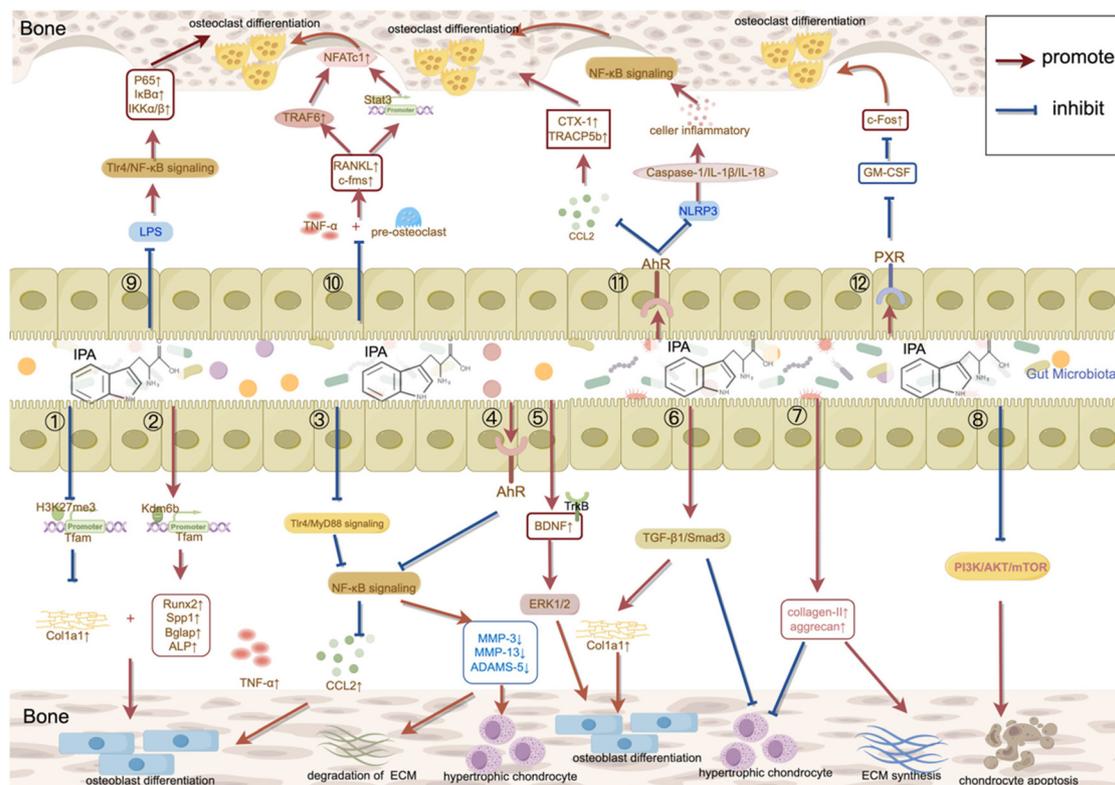
IL-37 inhibit osteoclast differentiation and promote osteogenic differentiation.<sup>82</sup> This intricate network of cells, cytokines, and factors maintains bone metabolism balance. The relationship between gut microbiota and bone homeostasis is well-established,<sup>1</sup> but the specific impact of the gut microbiota-derived metabolite IPA on bone metabolism remains underexplored.

## The effect of IPA on osteoblasts

Behera J *et al.* demonstrated that IPA plays a role in regulating osteogenic differentiation in epigenetic modifications. The overexpression of mitochondrial transcription factor (Tfam) in skeletal tissues promote the differentiation and mineralization of osteoblasts.<sup>83</sup> It was found that Histone 3 lysine 27 methylation 3 (H3K27me3) could down-regulate Tfam expression by inhibiting the transcriptional binding of Kdm6b/Jmjd3 histone demethylase at the Tfam promoter. IPA was shown to enhance osteoblast differentiation by increasing the binding of histone demethylase Kdm6b and reducing the binding of H3K27me3 to the Tfam promoter<sup>18</sup> (Fig. 1). Additionally, studies indicated that IPA treatment upregulates the expression of key genes involved in osteogenic differentiation such as Runx2, secreted phosphoprotein 1 (Spp1), Bglap2, ALP, and Col1a1 in osteoblasts to promote osteogenic differentiation.<sup>18,48</sup> Furthermore, administration of 0.1 mM IPA was shown to inhibit the Toll-like receptor 4/myeloid differentiation factor-88 (TLR4/MyD88) inflammatory signaling pathway, leading to the suppression of NF- $\kappa$ B signaling pathway activation. This inhibition resulted in the suppression of downstream inflammatory cytokines such as C-C motif chemokine 2 (CCL2) and TNF- $\alpha$ , ultimately leading to increased osteoblast differentiation<sup>57</sup> (Fig. 1).

IPA may influence osteoblast differentiation by regulating neurotrophic factors. In a randomized controlled trial, it was found that an increase in serum IPA levels following probiotic intervention was associated with serum brain-derived neurotrophic factor (BDNF) levels. This effect was mediated through the inhibition of pro-inflammatory factor production in microglial cells, exerting neuroprotective effects.<sup>84</sup> BDNF acts in an autocrine and paracrine manner to protect neurons, suggesting that increased expression of BDNF in neuronal cells may protect both the neurons themselves and surrounding neurons from inflammatory stimuli caused by activated microglial cells.<sup>85</sup> Accumulating evidence supports the involvement of BDNF in bone metabolism. It has been reported that BDNF promotes the mineralization of several highly differentiated cells *in vitro*.<sup>86,87</sup> Multiple studies have shown that BDNF binds to the TrkB receptor and activates the ERK1/2 signaling pathway, and activation of the ERK pathway in MSCs can promote their osteoblast differentiation.<sup>86,88</sup> Furthermore, BDNF combined with tricalcium phosphate and human BMSCs has been shown to induce ectopic osteogenesis related to neurogenesis in nude mice<sup>89</sup> (Fig. 1).

IPA also exerts biological effects through the TGF- $\beta$ 1/small mothers against decapentaplegic (Smad3) signaling pathway.



**Fig. 1** Effect of IPA on bone metabolism. ① IPA promoted osteoblast differentiation by decreased binding of H3K27me3 to the Tfam promoter. ② IPA promoted osteoblast differentiation by enhancing mitochondrial transcription activator Tfam *via* increased binding of histone demethylase Kdm6b. ③ IPA inhibits the TLR4/MyD88 signaling pathway and suppresses the expression of CCL2 and IL-1 $\beta$  to promote osteoblast differentiation. ④ IPA activates AhR to inhibit the expression of MMP-3, MMP-13, and ADAMS-5 induced by the NF- $\kappa$ B signaling pathway, resulting in reduced chondrocyte hypertrophy and degradation of ECM. ⑤ IPA enhances BDNF levels, facilitating its binding to the TrkB receptor and activating the ERK1/2 signaling pathway, thereby promoting osteoblast differentiation. ⑥ IPA upregulates the expression of Col1a1 to promote osteoblast differentiation and inhibits chondrocyte hypertrophy by activating the TGF- $\beta$ /Smad3 signaling pathway. ⑦ IPA upregulates the expression of collagen-II and aggrecan, promoting ECM synthesis and inhibiting chondrocyte hypertrophy. ⑧ IPA inhibits chondrocyte apoptosis induced by the PI3K/AKT/mTOR signaling pathway. ⑨ IPA inhibits osteoclast differentiation induced by LPS *via* the Tlr4/NF- $\kappa$ B signaling pathway by suppressing P65, I $\kappa$ B $\alpha$ , and IKK $\alpha$ / $\beta$  phosphorylation. ⑩ IPA inhibits the quantity of TNF- $\alpha$  binds to osteoclast precursors and subsequent osteoclast differentiation. ⑪ IPA exerts its effect by activating AhR: (1) IPA activates AhR to inhibit CCL2 expression and subsequent osteoclast differentiation; (2) IPA activates AhR to inhibit NLRP3 and the cell inflammation produced by NLRP3, which leads to osteoclast differentiation *via* the NF- $\kappa$ B signaling pathway. ⑫ IPA activates the RXR pathway to inhibit GM-CSF expression, resulting in increased expression of c-Fos and subsequent osteoclast differentiation. By Figdraw.

TGF- $\beta$ 1, a pivotal member of secreted polypeptide growth factors family, activates the Smad signaling pathway to regulate the proliferation, differentiation, and function of osteoblasts.<sup>90–92</sup> A study showed that IPA at a dose of 20 mg kg<sup>-1</sup> up-regulated the transcription and translation levels of Col1a1 in liver tissues *via* the TGF- $\beta$ 1/Smad3 pathway<sup>48</sup> (Fig. 1). Research suggests that the liver plays a role in carbohydrate and fat metabolism by secreting fibroblast growth factor 21 (FGF21),<sup>93</sup> BMP9,<sup>94</sup> and hepatokines,<sup>95</sup> which leads to changes in the quantity and structure of trabecular bone.<sup>96</sup> Col1a1 is one of the main proteins in the bone matrix and a key component in bone metabolism.<sup>97</sup> The role of liver-derived Col1a1 in bone metabolism remains unclear, but studies indicate that the liver may indirectly affect bone formation and remodeling through certain signaling pathways. Overexpression of liver-derived Col1a1 may be associated with alterations in bone mineralization. Excessive Col1a1 could

influence bone metabolism by modulating related cytokines (such as TGF- $\beta$ , TNF- $\alpha$ , IL-6, and RANKL) or promoting fibrosis, thereby disrupting the balance of bone metabolism.<sup>96–98</sup> Smad plays a direct role in the production and differentiation of osteoblasts and osteoclasts through the TGF- $\beta$  pathway in various contexts.<sup>90</sup> Studies have shown that stimulating the expression of Smad 2/3 mRNA, as well as promoting the secretion and synthesis of TGF- $\beta$ 1, can effectively enhance bone formation. TGF- $\beta$  not only induces the self-expression of osteoblasts but also increases the expression of BMP-2 in osteoblasts, thereby boosting their osteogenic potential.<sup>99</sup> Additionally, TGF- $\beta$  enhances the RANKL-mediated translocation of Smad 2 or Smad 3, forming a complex with Smad 4 that translocates into the nucleus, where it interacts with NFATc1 target genes to activate NFATc1 expression.<sup>100</sup> NFATc1 plays a critical role in regulating osteoclast differentiation. Studies have shown that embryonic stem cells lacking NFATc1

fail to differentiate into osteoclasts, and targeted disruption of NFATc1 in hematopoietic cells in mice leads to increased bone mass and a significant reduction in osteoclasts.<sup>101</sup> But the specific impact of IPA on bone metabolism *via* the TGF- $\beta$ 1/Smad3 pathway requires further investigation. While direct evidence linking IPA and osteoblasts is limited, there is a connection between inflammatory responses and osteoblast differentiation, suggesting a potential role for IPA in osteoblast differentiation by modulating inflammatory responses and other mechanisms. However, the precise underlying mechanisms warrant additional elucidation.

## The effect of IPA on osteoclasts

Previous studies have shown that IPA has an inhibitory effect on osteoclast differentiation by inhibiting the I $\kappa$ B/NF- $\kappa$ B pathway to reduce inflammatory factor recruitment.<sup>47,102</sup> The NF- $\kappa$ B family of transcription factors is activated downstream of RANKL, with the IKK complex initiating NF- $\kappa$ B activation in response to ligands like TNF- $\alpha$ , IL-1 $\beta$ , lipopolysaccharide (LPS), and RANKL.<sup>103,104</sup> Both catalytic IKK $\alpha$  and  $\beta$ , along with regulatory IKK $\gamma$ , promote osteoclast differentiation and lifespan by inducing various NF- $\kappa$ B subunits including P65, P50, and P52.<sup>105–107</sup> For instance, HFD can lead to intestinal LPS production, activating the TLR4/NF- $\kappa$ B signaling pathway and causing inflammatory damage, subsequently influencing the phosphorylation of P65, I $\kappa$ B $\alpha$ , and IKK $\alpha/\beta$ . In this context, IPA can inhibit TLR4/NF- $\kappa$ B signaling activity and reverse this inflammatory condition.<sup>21</sup> HFD also promotes osteoclasts formation and activity in bone marrow cells by increasing the levels of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ .<sup>108,109</sup> TNF- $\alpha$  binds to osteoclast precursors, leading to enhanced RANKL expression from bones. This chronic inflammatory signaling up-regulates c-fms, which recruits TRAF6 and induce increased expression of NFATc1,<sup>101</sup> facilitating osteoclast differentiation.<sup>110</sup> However, administration of IPA down-regulates the expression of osteoclast-related genes, including RANK and NFATc1 *in vitro*, reversing the HFD-mediated osteoclastogenesis.<sup>18</sup> RANKL also stimulates the expression of NFATc1 by activating the signal transducer and activator of transcription 3 (Stat3) pathway, which in turn controls the osteoclasts differentiation and bone homeostasis.<sup>111</sup> Studies have demonstrated that IPA can suppress Stat3 expression and phosphorylation.<sup>112</sup> Additionally, IPA supplementation reduces CCL2 expression *via* AhR.<sup>113</sup> CCL2 is known to promote osteoclasts fusion,<sup>114</sup> and deficient mice have reduced osteoclast-specific genes including NFATc1 and cathepsin K (CTX-1),<sup>115</sup> and result in increased bone mass and decreased bone resorption markers such as CTX-1 and tartrate-resistant acid phosphatase 5b (TRACP 5b)<sup>116</sup> (Fig. 1). Moreover, IPA activates AhR to inhibit nucleotide-binding oligomerization domain-like-receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation, thereby reducing procaspase-1 cleavage and IL-1 $\beta$  secretion.<sup>117</sup> The NLRP3 inflammasome exacerbates cellular inflammation through the

Caspase-1/IL-1 $\beta$ /IL-18 pathway,<sup>118</sup> which inhibits osteoblasts and promotes osteoclast differentiation *via* NF- $\kappa$ B activation.<sup>119–121</sup> AhR has been proven to negatively regulate NLRP3 inflammasome activity, with its agonist BaP inhibiting osteoclast differentiation and bone resorption by modulating the AhR-NF- $\kappa$ B pathway<sup>117,122,123</sup> (Fig. 1).

On the other hand, some studies have indicated that IPA may potentially promote the formation of osteoclasts. IPA has been shown to activate the PXR, leading to the suppression of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) expression.<sup>124</sup> Nevertheless, GM-CSF, known as a dendritic cell stimulating factor, has been found to strongly inhibit osteoclasts formation.<sup>125,126</sup> This inhibition occurs through GM-CSF acting on monocyte/macrophage precursor cells to reduce c-Fos levels and thus suppress osteoclast differentiation<sup>125</sup> (Fig. 1).

The contradictory results may be due to the activated signal pathways, and the osteoclasts differentiation stage. Given that research on the impact of IPA on osteoclasts is still in its nascent stages, further experimental validation is essential to establish a causal relationship between these factors.

## The effect of IPA on adipocytes

Adipogenesis also originates from MSCs, where osteoblasts and adipogenesis compete and have a reciprocal relationship.<sup>127</sup> Excessive fat accumulation leads to hypertrophy of adipocytes, which in turn enhances the expression of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL6 and inducible nitric oxide synthetase (iNOS) in adipose tissue. These cytokines are then released into the bloodstream, promoting a low-grade systemic inflammation or the expression of the pro-osteoclastogenic factor RANKL, leading to osteoclast differentiation. This disrupts the balance of bone remodeling and dysregulate bone homeostasis.<sup>128–132</sup> IPA at a dosage of 20 mg kg<sup>-1</sup> can reduce fat accumulation. Supplementation with type 2 resistant starch, a probiotic that significantly increases IPA concentration in mouse plasma, can reduce liver fat accumulation and fat cell size in white adipose tissue, thereby improving fat metabolism abnormalities in obese mice.<sup>133</sup> Studies by Tao Yan *et al.* showed that serum IPA concentration decreased in HFD-fed mice.<sup>134</sup> Additionally, orally administered IPA at a dosage of 100 mg kg<sup>-1</sup> significantly reduced the transcription of sterol regulatory element-binding protein 1c (SREBP1c) and fatty acids in HFD mice.<sup>135</sup> Metabolomics analysis has demonstrated decreased levels of IPA in obesity.<sup>136</sup> Following gastric bypass surgery, oral administration of IPA at 20 mg kg<sup>-1</sup> resulted in continuous weight loss by regulating the increase in intestinal permeability and reversing the interferon gamma-induced (IFN- $\gamma$ ) transcriptional increase in the expression of the fructose transporter SLC2A5 (GLUT5).<sup>54,137</sup> HFD feeding activates the NF- $\kappa$ B pathway, leading to inflammatory injury, which can be reversed by IPA.<sup>21</sup>

Moreover, research has shown that IPA supplementation can reduce macrophage infiltration in apolipoprotein E-deficient (ApoE<sup>-/-</sup>) mice.<sup>138</sup> Apolipoprotein E (ApoE), a lipoprotein, is involved in fat accumulation in bone and other organs, as well as in regulating bone metabolism. In ApoE<sup>-/-</sup> mice, there is an increase in lipogenic markers such as peroxisome proliferator-activator receptor gamma (PPAR $\gamma$ ), fatty acid binding protein4 (FABP4) and Perilipin2 (RLIN2), while the expression of osteogenic markers like RUNX2, osteopontin (OPN), and OCN is significantly reduced.<sup>139</sup> PPAR $\gamma$  is known to play a crucial role in macrophage activation and, along with the CAAT enhancer binding proteins (C/EBPs) family, is a key transcription factor for adipogenic differentiation.<sup>140</sup> The upregulation of PPAR $\gamma$  leads to increased macrophage infiltration, adipogenesis, bone resorption, and osteoclasts differentiation.<sup>141,142</sup>

## The effect of IPA on chondrocytes

Chondrocytes, originated from BMSC, undergo a series of differentiation processes to develop into hypertrophic chondrocytes. These hypertrophic chondrocytes represent the final state of growth plate chondrocytes and can potentially lead to degenerative maturation, resulting the imbalances and skeletal diseases.<sup>143</sup> Healthy chondrocytes typically express type II collagen (COL2), aggrecan and SRY-Box transcription factor 9 (SOX9),<sup>144</sup> while chondrocytes under inflammatory conditions elevated levels of matrix metalloproteinase (MMP) -3, MMP-13, and thrombospondin motifs-5 (ADAMTS-5). The excessive release of these enzymes can degrade the chondrocyte extracellular matrix (ECM) and disrupt the normal chondrocyte phenotype.<sup>145</sup> Studies have shown that IPA can mitigate chondrocyte aging and inflammation by acting on the AhR/NF- $\kappa$ B axis. IPA demonstrates strong anti-inflammatory properties and inhibits the NF- $\kappa$ B signaling pathway by activating the AhR in chondrocytes, thereby reducing the inflammatory effects induced by IL-1 $\beta$  on cytokines like TNF- $\alpha$ , IL-6, COX-2, and iNOS, as well as decreasing the expression of catabolic factors such as MMP-3, MMP-13 and ADAMTS-5 in chondrocytes.<sup>146</sup> Additionally, IPA injections have been found to enhance the expression of aggrecan and collagen-II, key components of the chondrocyte ECM, promoting matrix synthesis, suppressing hypertrophic differentiation, and maintaining a normal chondrocyte phenotype<sup>146</sup> (Fig. 1).

IPA also can activate the TGF- $\beta$ /Smad signaling pathway by phosphorylating Smad3 after intervention.<sup>48</sup> TGF- $\beta$  binds to type I receptors activin-like kinases (ALK) 1 and ALK5, leading to the phosphorylate of Smad3, which in turn inhibits chondrocyte hypertrophy and prevents terminal differentiation<sup>147,148</sup> (Fig. 1).

Studies have shown that IPA treatment inhibits the activation of the PI3K/AKT/rapamycin (mTOR) signaling pathway.<sup>49</sup> The PI3K/AKT signaling pathway is an essential regulator of chondrocyte survival and apoptosis, which can also minimize cartilage degeneration by preventing chondrocyte

apoptosis. However, there are researches demonstrated that mTOR knockout in cartilage could reduce apoptosis and alter cartilage homeostasis in mice and the up-regulated mTOR along with the increased expression of pro-inflammatory cytokines such as IL-1 $\beta$ , which increases in chondrocytes apoptosis<sup>149,150</sup> (Fig. 1). However, further research is needed to determine if IPA can restore cartilage homeostasis by inhibiting this pathway.

## The effects of IPA on bone disease

### Osteoporosis (OP)

OP is a systemic bone disease characterized by low bone mass, damage to the microstructure of bone tissue, increased bone fragility and susceptibility to fractures.<sup>151</sup> OP can be classified into primary OP and secondary OP. Primary OP includes postmenopausal osteoporosis (PMOP), senile OP and idiopathic OP, while secondary OP refers to OP caused by any disease or medication affecting bone metabolism.<sup>152,153</sup> In physiological conditions, bone formation by osteoblasts and bone resorption by osteoclasts maintain the remodeling of bone tissue. However, an imbalance between osteoblasts and osteoclasts can lead to accelerated deterioration of bone tissue, bone loss, and ultimately osteoporosis.<sup>151</sup>

Currently, postmenopausal women, older men, diabetes patients, and obese individuals are considered high-risk populations for OP, with different mechanisms leading to the development of OP in each population.<sup>152</sup> In postmenopausal women, a gradual decrease in estrogen levels reduces binds to estrogen receptor alpha (ER $\alpha$ ) on the surface of osteoclasts, enhancing osteoclast differentiation.<sup>154</sup> Older men lack essential nutrients such as calcium and vitamin D required for bone matrix formation, resulting in decreased bone mineralization and increased bone fragility.<sup>155</sup> Additionally, the decline of gonadal function and sex hormone levels in the elderly are closely related to the decrease in bone mass and bone mineral density.<sup>156</sup> In diabetic patients, disrupted glucose metabolism leads to a high catabolic state and excessive urinary calcium excretion, inhibiting bone tissue formation.<sup>157</sup> Excess fat accumulation in obese individuals induces an imbalance between bone marrow adipocytes and osteoblasts, leading to OP.<sup>158</sup>

IPA been found to have a regulatory effect on osteoporosis caused by obesity. Sofia Cussotto *et al.* demonstrated a significant decrease in IPA levels in obese patients, but noted that after gastric bypass surgery, IPA led to continuous weight loss in these patients.<sup>159</sup> Animal studies have shown that HFD mice can decrease bone formation and reduce mechanical bone quality.<sup>108</sup> Conversely, oral supplementation of IPA to HFD-fed obese mice has been found to increase trabecular bone formation and improve cortical bone mechanical strength.<sup>18</sup> Additionally, IPA has been shown to regulate dyslipidemia *in vitro*.<sup>135</sup> Obesity can lead to hyperlipidemia, which in turn can negatively impact bone health. Elevated levels of total cholesterol (TC) and low-density lipoprotein cholesterol

(LDL-c) have been associated with lower bone mass and increased fracture risk, as they inhibit osteoblast differentiation, promote osteoclast differentiation, and lead to bone loss.<sup>160</sup> Interestingly, IPA has been shown to significantly decrease serum levels of TC, LDL-c, and total triglyceride (TG).<sup>135</sup> Furthermore, hyperlipidemia can trigger chronic systemic inflammation, which can reduce osteoblast activity and enhance osteoclast activity by increasing levels of pro-inflammatory cytokine. These effects are believed to be the main contributors to inflammation-induced bone loss. While direct evidence is limited, research suggests that IPA may lower pro-inflammatory cytokine levels, potentially indirectly regulating inflammation-induced bone loss.

Oxidative stress (OS) occurs when the balance between oxidation and antioxidant function is disrupted in the body, leading to increased oxidation. This imbalance can activate the NF- $\kappa$ B signaling pathway, resulting in the production of pro-inflammatory cytokines, that contribute to bone loss.<sup>161</sup> Obesity often leads to higher levels of reactive oxygen species (ROS), which stimulate the secretion of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) in osteoclasts and osteoblasts. These cytokines play a role in the development of osteoporosis by enhancing osteoclast function and inhibiting osteoblast function.<sup>162</sup> ROS and antioxidant enzymes help regulate the redox balance, with ROS inhibiting osteogenic activity by suppressing the expression of Runx2 and *osx*, and disrupting bone remodeling.<sup>163</sup> Meanwhile, ROS can prevent mitochondrial apoptosis of osteoblasts. By increasing mitochondrial membrane permeability, releasing cytochrome c into the cytoplasm to interact with Apaf1, ultimately increasing the expression of caspase apoptotic proteins.<sup>164</sup> Glutathione (GSH) metabolism and ferroptosis are involved in ROS-mediated regulation of bone metabolism.<sup>165</sup> GSH can promote osteoblastic differentiation by increase the expression of Col1a1, OCN and ALP.<sup>166</sup> Furthermore, increasing GSH levels can inhibit the ROS/NF- $\kappa$ B signaling pathway, subsequently suppressing RANKL-mediated osteoclastogenesis.<sup>167</sup> Recent research suggests that IPA may have a role in OP by acting as an anti-oxidation and anti-inflammation agent. IPA, a potent antioxidant with no pro-oxidant activity, protects cells from oxidative damage and lipid peroxidation induced by ROS and iron(III) chloride.<sup>20,168,169</sup> It has been reported that IPA enhances redox-regulatory systems by increasing the activity of Acetylcholinesterase (AChE) and antioxidant enzymes, as well as GSH levels, while also attenuates the activity of caspase-9 and -3, and the expression of proinflammatory cytokines.<sup>170</sup> Besides, studies have confirmed a positive correlation between iron overload and the development of OP, with it being one of the driving factors of postmenopausal OS.<sup>171,172</sup> Excessive iron binds with transferrin to form Fe<sup>3+</sup>, which can lead to increased production of free radicals.<sup>173</sup> Iron ions have been shown to stimulate the differentiation of osteoclasts and promote bone resorption by generating ROS.<sup>174</sup> Animal experiments have demonstrated that co-incubated IPA can prevent the decrease in cell membrane fluidity caused by Fe<sup>3+</sup>.<sup>169</sup> Moreover, IPA inhibit lipid peroxidation damage in hamster testes caused by iron ions,<sup>175</sup>

suggesting a potential role in protecting cell membranes from iron-induced oxidative damage. Furthermore, obesity is known to induce systemic OS by increasing ROS production, suppressing antioxidant enzymes expression, and promoting the secretion of pro-inflammatory cytokines, which exacerbate inflammatory responses.<sup>176,177</sup> OS leads to the conversion of oxygen into ROS, upregulating pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6.<sup>178</sup> The accumulation of ROS activates the NF- $\kappa$ B signaling pathway, enhances NLRP3 inflammasome synthesis, and triggers the release of inflammatory cytokines.<sup>179</sup> These cytokines can stimulate osteoclast bone resorption by modulating the RANK/RANKL/OPG axis in obesity. In contrast, IPA has shown promise in reducing ROS accumulation, suppressing NF- $\kappa$ B signaling pathway activation, and mitigating inflammatory responses associated with obesity.<sup>20,21</sup> However, further research is necessary to fully understand how IPA modulates osteoporosis through oxidation–reduction mechanisms.

Furthermore, IPA may also influence bone loss through neural regulation. *In vitro* experiments have shown that 500  $\mu$ M IPA can reverse angiotensin II (Ang II)-induced endothelial cell apoptosis by promoting AKT phosphorylation.<sup>180</sup> Ang II is an essential component of the hypothalamic endocrine system, playing a key role in regulating downstream hormone secretion and autonomic functions, which are involved in bone metabolic homeostasis and the development of bone-related diseases.<sup>181</sup> Ang II activates the sympathetic nervous system, promoting the release of catecholamines such as norepinephrine, which ultimately leads to inhibited osteogenesis and enhanced bone resorption.<sup>182</sup> Ang II antagonists can alleviate ovariectomy-induced osteoporosis by counteracting central renin–angiotensin system activity.<sup>182</sup> Furthermore, Ang II may influence bone metabolism and fracture healing by regulating vascular tone and bone perfusion.<sup>182</sup> However, further investigation is needed to clarify how IPA induces the regulation of bone loss *via* Ang II.

Although limited research exists on the effects of IPA on osteoporosis, its notable impact on anti-inflammation, anti-oxidation, neuroregulatory, and metabolism enhancement has garnered increasing interest. It is essential to integrate existing knowledge on osteoporosis, gut microbial metabolites, biomarker research, nutritional intervention, and drug development to advance early disease prevention, diagnosis, and treatment in clinical practice.

### Rheumatoid arthritis (RA)

RA is a chronic inflammatory autoimmune disease characterized by persistent tenderness and articular synovitis.<sup>183</sup> Early research suggests that the concentrations of IPA were decreased in the RA mice model, but were restored after treatment with Wu-tou decoction.<sup>184</sup> Clinical investigations by Heng Fang *et al.* revealed that the expression of NLRP3 inflammasome is elevated in RA patients, while administration of IPA has shown to suppress NLRP3 inflammasome activation.<sup>117</sup> Nonetheless, there is insufficient direct evidence to confirm the clinical effectiveness of IPA in treating RA, thus further research is warranted.

Multiple theories exist regarding the pathogenesis of rheumatoid arthritis. Inflammatory cytokines, immune cells, and synovial fibroblasts could activate osteoclasts, inhibit osteoblasts, induce bone destruction, bone loss, and promote RA development.<sup>185–187</sup> T cells, particularly T helper (Th17) and Fox3<sup>+</sup>T regulatory cells (Treg) cells, are abundant in RA synovium and play crucial roles in inflammatory processes and bone loss.<sup>188</sup> Th17 cells regulate osteoclastogenesis through the RANKL/RANK/OPG pathway, promoting RA progression.<sup>189</sup> On the other hand, Treg cells suppress RANKL and M-CSF levels, inhibiting osteoclast differentiation and promoting osteoblast differentiation, potentially delaying RA development.<sup>190</sup> Notably, IPA has been reported to influence T cell differentiation. Clinical studies have revealed a negative correlation between Th17 cell abundance and IPA concentration in healthy adults.<sup>191</sup> While direct evidence is lacking, research suggests that IPA treatment might *via* activated AhR induce the methylation of the IL-17 promoter and partial demethylation of the FoxP3 promoter, thereby reversing the significant reduction of Tregs and the marked increase in Th17 numbers in a mouse model of disease condition.<sup>192,193</sup> This implies that IPA may indirectly regulate osteoclasts differentiation and the occurrence of RA induced by Th17. In addition, Th cells secrete pro-inflammatory cytokines like IL-17, IFN- $\gamma$  and TNF- $\alpha$ , which can upregulate RANKL expression, affects the RANK-RANKL-OPG pathway and thereby activates osteoclasts and the RA immune response, while Tregs inhibit various Th cell-mediated proinflammatory immune responses by release anti-inflammatory cytokines.<sup>194,195</sup> Supplementing with IPA can enhance Treg differentiation and inhibit Th cells production of pro-inflammatory factors.<sup>192</sup> Research has found that marrow-derived suppressor cells (MDSCs) accumulate in the spleens of mice with collagen-induced arthritis during the peak of arthritis progression, where they inhibit the proliferation and differentiation of CD4<sup>+</sup> T cells. CD4<sup>+</sup> T cells can differentiate into TH17 cells that produce pro-inflammatory cytokines, contributing to the occurrence of RA.<sup>196</sup> IPA supplementation has been found to mediate AhR activation to enhance the suppressive function of MDSCs.<sup>197</sup> Nevertheless, there is no direct evidence to prove that IPA can delay the progression of RA by enhancing the suppressive effects of MDSCs, indicating the need for further research. Furthermore, one study reported that IPA activated AhR to suppress the expression of MMP genes,<sup>46</sup> enzymes produced by synovial fibroblasts that contribute to cartilage destruction in RA.<sup>198</sup> While there is limited research on the effects of IPA on RA, animal studies suggest that IPA may potentially mitigate the onset of RA by regulating the generation of inflammatory factors and the degradation of the extracellular matrix.

### Osteoarthritis (OA)

OA is a degenerative joint disease characterized by progressive cartilage degradation, synovial membrane inflammation, osteophyte formation and subchondral bone sclerosis. Its onset is related to multiple changes in the proliferation, viability and secretion characteristics of chondrocytes.<sup>199</sup> Factors

such as aging, injury, genetics and obesity can trigger local low-grade inflammation in joints, leading to damage in OA cartilage.<sup>200,201</sup> In normal conditions, articular cartilage of a regulated ECM that is produced by chondrocytes, the sole cell type present in cartilage tissue.<sup>202</sup> However, inflammatory cytokines, oxidative stress, and adipokines have the potential to induce chondrocyte differentiation and promote the development of OA.<sup>199</sup>

IPA may play a crucial role in delaying the development and progression of OA. Through animal experiments, Zanzhu Li *et al.* observed a lower concentration of IPA in the OA group compared with the sham operation group.<sup>203</sup> The inflammatory cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$  are highlighted as the most secreted cytokines in OA, known for their pro-inflammatory effects and ability to induce loss of chondrocyte phenotype through various signal pathways.<sup>204</sup> IPA administration could decrease the expression of these inflammatory cytokines by suppressing the NF- $\kappa$ B signaling pathway, thus slowing down OA progression.<sup>46</sup> In OS, iNOS can produce nitric oxide (NO) leading to chondrocyte apoptosis and inhibition of chondrogenic phenotype.<sup>205,206</sup> Supplementation with IPA decreases the expression of iNOS in OA.<sup>46</sup> Additionally, IPA has demonstrated the ability to counteract the effects of IL-1 $\beta$  on genes related to matrix degradation, such as MMP-3, MMP-13, and ADAMTS-5, through the AhR/NF- $\kappa$ B signaling pathway, thereby reducing ECM degradation and promoting matrix synthesis.<sup>46</sup> Furthermore, the cartilage degradation and synovial inflammation were attenuated after IPA treatment. Moreover, IPA can inhibit the expression and phosphorylation of Stat3.<sup>112</sup> Latourte *et al.* demonstrated the activation of Stat3 in chondrocytes that induces cartilage destruction and osteophyte formation in OA.<sup>207</sup> It is worth mentioning that the effects of IPA on other endogenous ligands and signaling pathways in OA, apart from the AhR/NF- $\kappa$ B signaling pathway, have not been validated and will become an essential subject of future research.

In OA, ROS are typically produced at low levels in chondrocytes and play a role in intracellular signaling pathways that regulate cartilage metabolism.<sup>208</sup> However, in OA chondrocytes, the increased production of ROS lead to the degradation of ECM components and trigger chondrocyte death.<sup>209,210</sup> IPA has been shown to protect cells from OS damage, reducing the expression of proinflammatory cytokines.<sup>20,21,57,168</sup> Various experiments have demonstrated that ROS generated by OS can impact the activity of osteoblasts, resulting in decreased bone mineralization and bone loss.<sup>211,212</sup> Excessive ROS can inhibit osteoblast activation, preventing the production of OPG and promoting osteoclast differentiation and bone resorption.<sup>212</sup> The depletion of antioxidants can accelerate bone loss by activating proinflammatory cytokines like TNF- $\alpha$ .<sup>213</sup> Research has indicated that administering IPA at a dose of 25 mg kg<sup>-1</sup> can enhance redox-regulatory systems by boosting the activities of AChE and antioxidant enzymes, while also attenuating the activities of caspase-9 and -3.<sup>170</sup> However, *in vitro* studies have revealed that treating cells with 5  $\mu$ g mL<sup>-1</sup> of IPA can increase intracellular Ca<sup>2+</sup> concentration and ROS production.<sup>214</sup>

Despite these findings, there is currently insufficient direct evidence to conclusively determine the impact of IPA on bone metabolism in the context of OS, necessitating further investigation.

## Conclusion and perspective

With the rapid advancement of metabolomics, the role of gut microbiota metabolites in regulating host bone health and disease has gradually come to light. IPA, an important metabolite derived from gut microbiota, has been shown to be closely associated with the occurrence of bone metabolic diseases. Previous studies have established links between IPA and various metabolic conditions, such as obesity and diabetes. Experimental evidence further suggests that IPA intervention can effectively reduce body weight, peripheral fat levels, pro-inflammatory cytokine expression, and oxidative stress, thereby exerting profound impacts on host health.

In the context of bone metabolism, IPA demonstrates significant anabolic effects. Research shows that IPA promotes osteoblast differentiation by upregulating mitochondrial Tfam, a mechanism that plays a key role in preventing bone loss.<sup>18</sup> Additionally, IPA modulates the host's immune response and inflammation levels by activating ligands such as the AhR and PXR, thus providing indirect protection to bone.<sup>46,47</sup> Although the mechanisms underlying IPA's role in bone metabolism are not yet fully understood, existing evidence highlights its critical functions in regulating inflammation, immune response, and oxidative stress.

The findings of this study suggest that IPA holds substantial potential as a therapeutic molecule for bone metabolic diseases. Its effects in reducing inflammation, promoting osteoblast differentiation, and preventing bone loss position it as a promising candidate for treating conditions like osteoporosis. Future research could focus on further elucidating the molecular mechanisms of IPA in bone metabolism, particularly through preclinical and clinical trials. Moreover, examining the impact of different doses and routes of IPA administration on bone metabolism will help optimize its efficacy and safety in clinical applications. These findings not only enhance our understanding of IPA's role in bone health but also offer new directions and strategies for the therapeutic development of bone metabolic diseases.

## Author contributions

Huimin Xu: conceptualization, writing – original draft. Yingzhe Luo: investigation, resource, writing – review & editing. Yi An: supervision, writing – review & editing. Xi Wu: supervision, funding acquisition, writing – review & editing.

## Data availability

No data was used for the research described in the article.

## Conflicts of interest

No potential conflicts of interest were disclosed.

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