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Review article

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NEW INSIGHTS INTO TRYPTOPHAN AND ITS METABOLITES IN THE REGULATION OF BONE METABOLISM

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Osteoporosis, a debilitating disease caused by an imbalance between the action of osteoblasts and osteoclasts, is becoming an increasing problem in today's aging population. Although many advances in this field have addressed certain aspects of disease progression and pain management, new approaches to treatment are required. This review focuses on the influence of tryptophan, its metabolites and their influence on bone remodeling. Tryptophan is a precursor to serotonin, melatonin, kynurenines and niacin. Changes of tryptophan levels were noticed in bone metabolic diseases. Moreover, some works indicate that tryptophan plays a role in osteoblastic differentiation. Serotonin can exert different effects on bones, which depend on site of serotonin synthesis. Gut-derived serotonin inhibits bone formation, whereas brain-derived serotonin enhances bone formation and decreases bone resorption. Melatonin, increased differentiation of human mesenchymal stem cells into the osteoblastic cell lineage. Results of melatonin action on bone are anabolic and antiresorptive. Activation of the second tryptophan metabolic pathway, the kynurenine pathway, is associated with osteoblastogenesis and can be implicated in the occurrence of bone diseases. Oxidation products like kynurenine stopped proliferation of bone marrow mesenchymal stem cells. This may result in inhibition of osteoblastic proliferation and differentiation. Kynurenic acid acts as an antagonist at glutamate receptors, which are expressed on osteoclasts. Quinolinic acid activates N-methyl-D-aspartate receptors. 3-hydroxyanthranilic acid exhibits pro-oxidant and antioxidant activity. Decreased concentration of 3-hydroxyanthranilic acid can be one of the causes of osteoporosis. 3-hydroxykynurenine reduced the viability of osteoblast-like cells. Picolinic acid exerted osteogenic effect *in vitro*. Kynurenine derivatives exert various effects on bones. Discovery of the exact mechanism of action of tryptophan metabolites on bones may take us a step closer to understanding the complicated mechanism of bone metabolism, which in turn may result in finding a new, effective therapy for treating bone diseases.

Key words: *tryptophan, melatonin, serotonin, kynurenine, bone remodeling, osteoporosis, osteoblast*

INTRODUCTION

Bone remodeling is a process that occurs throughout a person's entire life. Osteoblasts, necessary for this process, are responsible for forming new bone tissue while osteoclasts take part in bone resorption. This complex process is regulated by many factors, some of which are still not well understood. An imbalance between osteoblast and osteoclast action leads to many metabolic diseases, of which osteoporosis is most well-known (1).

The cause of osteoporosis is the above-mentioned imbalance - excessive bone resorption and insufficient bone formation, which leads to increased risk of fractures and significant reduction in quality of life (1, 2). Taking into account increasing life expectancy and an ageing population, it can be reasonably concluded that osteoporosis is, and will continue to be, a growing public health problem.

Tryptophan (TRP), one of the 22 standard amino acids isolated in 1901, belongs to the group of exogenous amino acids. This means that it cannot be synthesized by organisms, and must be provided in the diet. TRP plays an important role in organisms, for instance being a precursor to compounds such as serotonin, melatonin, niacin and kynurenine derivatives (3-6).

The serotonin biosynthesis pathway begins with converting L-tryptophan into 5-hydroxy-tryptophan. The principal enzyme of this conversion is tryptophan hydroxylase (Tph). The next and last step of serotonin biosynthesis is decarboxylation catalyzed by L-amino acid decarboxylase (7) (*Fig. 1*).

Melatonin (N-acetyl-5-methoxytryptamine), which controls circadian rhythm, is synthesized *via* the serotonin pathway. To synthesize melatonin, it is necessary to convert serotonin to N-acetyl-5-hydroxytryptamine. This step is carried out with the enzyme N-acetyltransferase, which is activated in darkness. The final enzyme, 5-hydroxyindole-O-methyltransferase converts N-acetyl-5-hydroxytryptamine into melatonin (8, 9) (*Fig. 1*).

The second tryptophan metabolic pathway is the kynurenine pathway, which leads to the production of many active metabolites. The end product of this pathway is nicotinamide adenine dinucleotide (NAD) (*Fig. 2*). NAD is a coenzyme in redox reactions, which also fulfills many other meaningful functions in organisms. The kynurenine pathway of TRP degradation is linked to the occurrence of many neurodegenerative, psychiatric, and

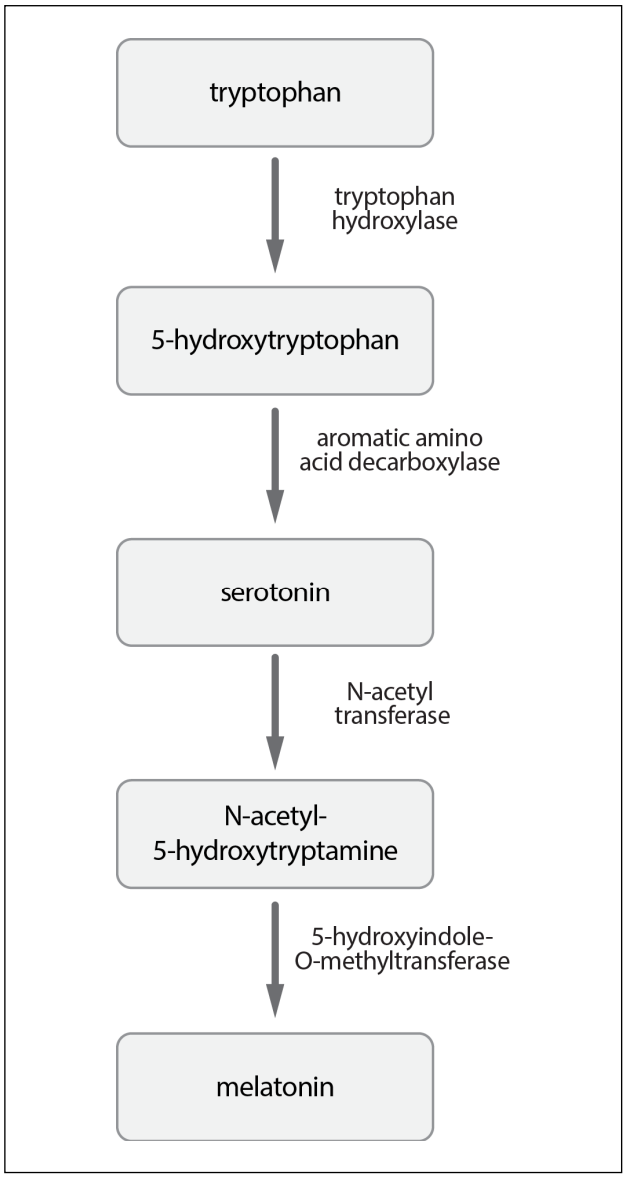

Fig. 1. The synthesis of serotonin and melatonin from tryptophan.

inflammatory diseases, cancer, as well as and disorder associated with HIV. Moreover, activation of the kynurenine pathway is associated with osteoblastogenesis, which can be implicated in the occurrence of bone diseases (6, 10-12).

This review focuses on tryptophan and its metabolic products - serotonin, melatonin and kynurenine derivatives, and their influence on bone metabolism. The authors would particularly like to highlight the issue regarding the kynurenine pathway in the view of bone remodeling, as the least known pathway in that question, but with interesting influences on bones.

TRYPTOPHAN

Tryptophan in male idiopathic osteoporosis

Coming back to the crucial compound for the serotonin and kynurenines synthesis pathway - TRP, changes in its level were observed in various conditions. There are findings showing changes in TRP concentrations in bone metabolic diseases. Pernow *et al.* examined changes in amino acids including TRP in men with idiopathic osteoporosis (13). Differences between postmenopausal osteoporosis and male idiopathic osteoporosis (MIO) are noticeable in microstructural abnormalities. There is also evidence of osteoblast dysfunction and decreased bone formation in MIO. Bone resorption, which is clearly increased in postmenopausal osteoporosis, can be normal or only slightly increased in MIO. Measured levels of TRP in erythrocytes in MIO were lower than in controls and correlated positively with femoral neck and lumbar spine bone mineral density as well as histomorphometry variables of bone formation (13, 14).

Tryptophan in alcohol induced osteoporosis

The involvement of TRP in other osteoporosis models, specifically alcohol induced osteoporosis, was examined by Pallu *et al.* (15). They used synchrotron ultraviolet microspectroscopy on rat cortical bone because they observed of an excess apoptosis in osteocytes. Synchrotron UV microspectroscopy allows for an evaluation of osteocyte metabolism. They assayed tryptophan/collagen ratios and noticed that it was dependent on alcohol dose. Differences were significant between moderate and high alcohol consumption samples in the tryptophan/collagen ratio. Considering the relation of TRP and serotonin, measurement of this ratio could give data related to serotonin metabolism. Significant differences in the ratio dependent on alcohol dose, indicate that tryptophan metabolic pathway is connected with osteocyte response to high alcohol consumption (15).

Tryptophan and aging

The prevalence of osteoporosis is highest among the aging population. The correlation between higher age and osteoporosis can be attributed to the aforementioned increased levels of oxidation products from TRP, as well as augmented numbers of reactive oxygen species (ROS). ROS can influence generation and survival of bone cells, which may be a visible in link between oxidative stress and a decrease in bone mineral density (16, 17). The pathogenic age-dependent impact of oxidized metabolites on bone resulting in decreasing its mass was acknowledged by El Rafaey *et al.* (18) (*Table 1*). Unmodified TRP activates anabolic signaling pathways in bone marrow mesenchymal stem cells (BMMSCs). Data indicate that L-tryptophan stimulated the proliferation of BMMSCs, increased expression of osteocalcin and the alkaline phosphatase protein, which is considered a marker of differentiation. All these elements signal that L-tryptophan plays a role in osteoblastic differentiation. In contrast, oxidize products stopped proliferation of BMMSCs, which may result in inhibition of osteoblastic proliferation and differentiation leading to bone loss (18) (*Fig. 3*).

Oxidation products like kynurenine are formed due to TRP degradation through the kynurenine pathway. The levels of these oxidation products are dependent on age. Braidy *et al.* noticed a drop in both TRP and indoleamine 2, 3-dioxygenase (IDO) levels with age in all tissue (19). Indoleamine 2, 3-dioxygenase activity in the liver and kidney also decreased with age. Activity of IDO in the brain, however, increased. These observations are in agreement with the findings of kynurenine enhancement and reduction of TRP in the brain (19).

SEROTONIN PATHWAY

Serotonin

A primary rate-limiting factor in serotonergic functioning is tryptophan hydroxylase (Tph) enzyme (*Fig. 1*). Until 2003, there was no evidence for existance of more than one isoform of Tph.

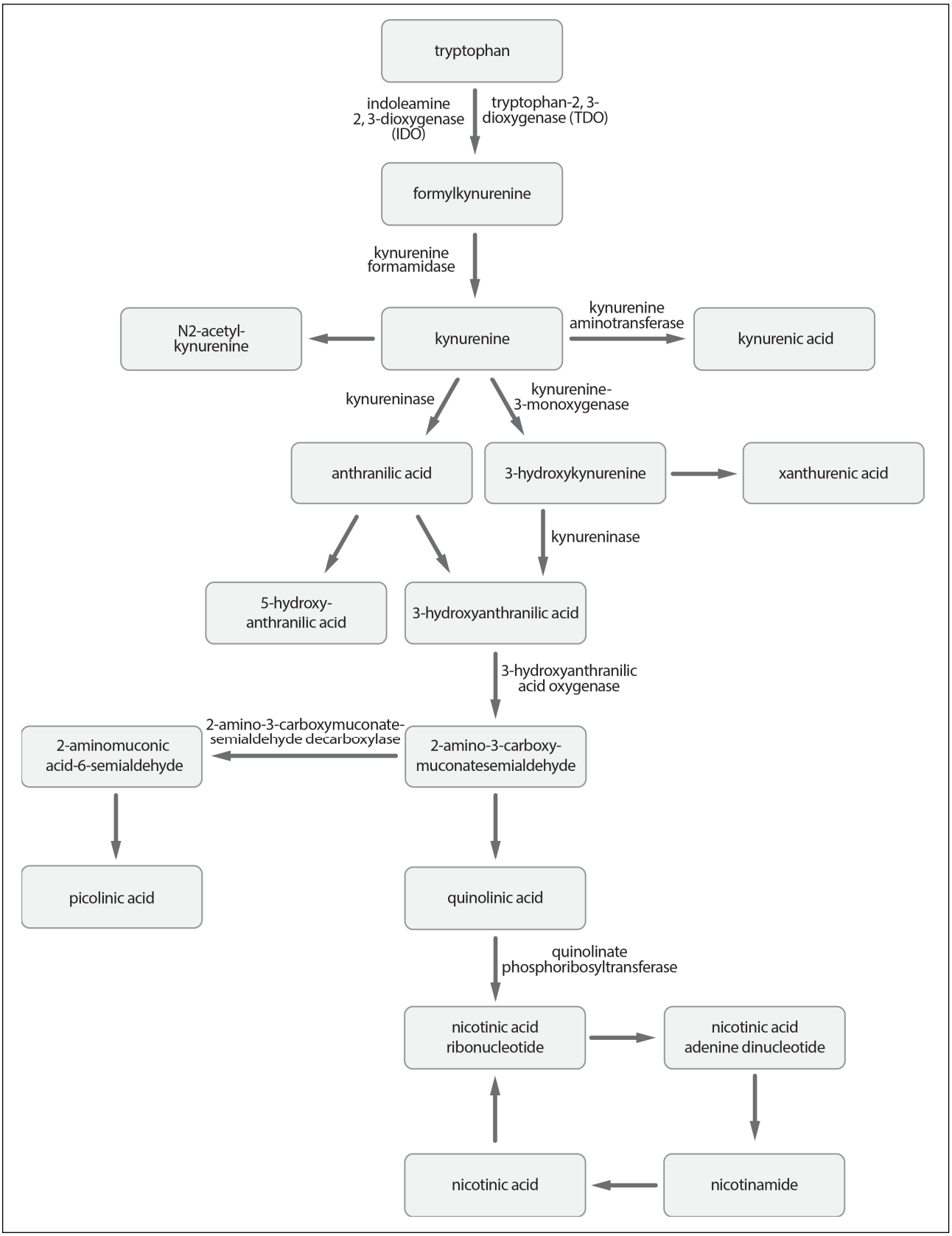

Fig. 2. The kynurenine pathway.

That same year, Walther *et al.* proved, by using the Tph knockout murine model, that Tph exists in two isoforms (20). During the experiment Tph KO mice exhibited a normal level of brain serotonin and were deficient in serotonin on the periphery. This revelation led to the discovery of a new gene, called tryptophan hydroxylase 2 (Tph-2), which encodes the second

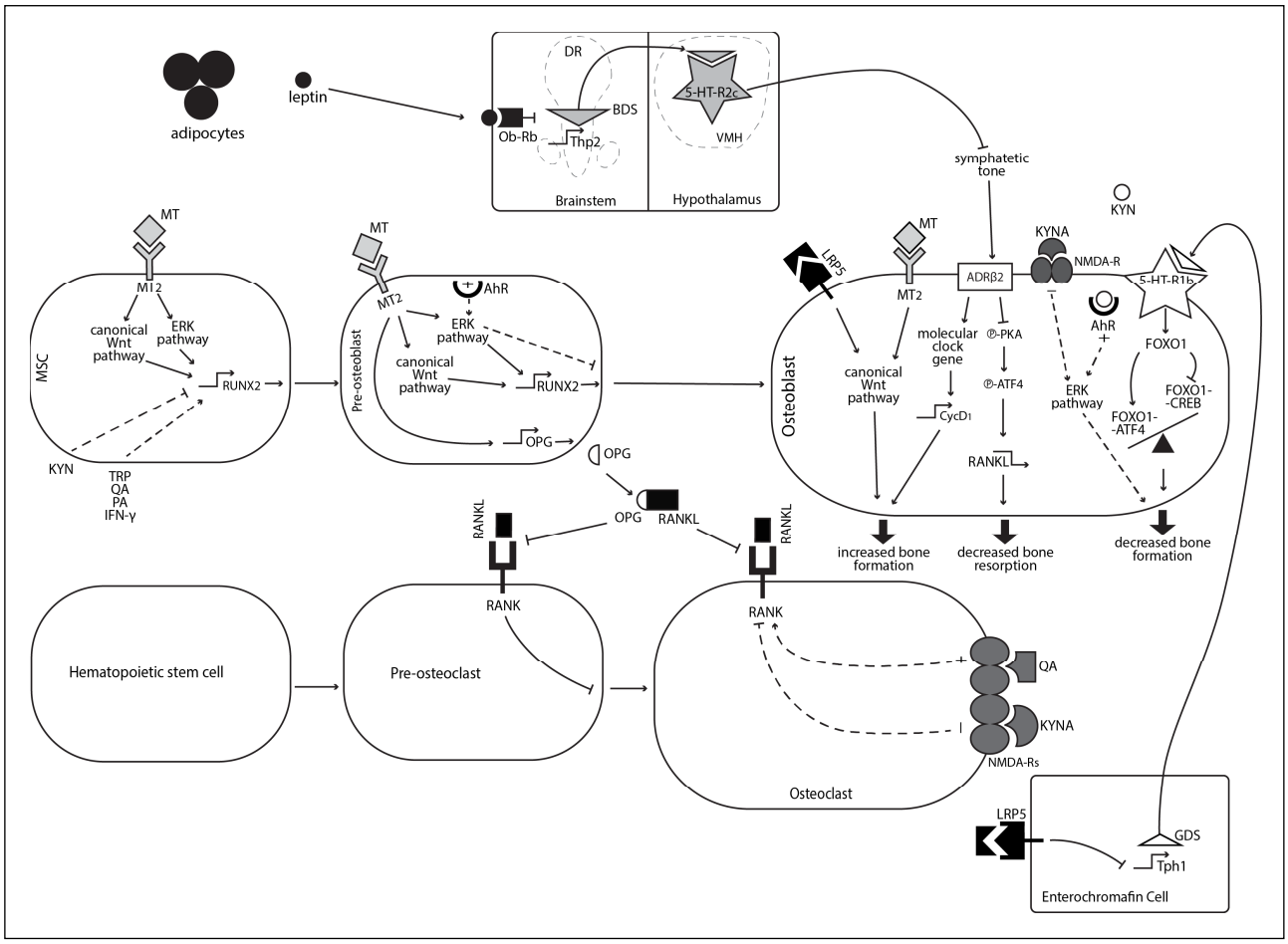

Fig. 3. Influence of serotonin and kynurenines on bone cells. BDS enhances bone formation and decreases bone resorption. BDS activates 5-HT-R_{2c} in VMH what leads to decreased sympathetic tone in osteoblasts. Results of this action are decreased synthesis of RANKL triggered by inhibition of PKA/ATF4 pathway, and increased osteoblasts' proliferation connected with molecular clock gene/cyclin D1 cascade. Leptin can inhibit synthesis of BDS. LRP-5 through canonical Wnt pathway can increase osteoblasts' proliferation and by inhibiting Tph-1 can suppress GDS synthesis. GDS inhibits bone formation by blocking osteoblasts' proliferation through FOXO1 mechanisms. Kynurenine derivatives exert multi-faces effect on bones. Decreased bone formation can be caused by KYN. KYN *via* RUNX2-related mechanisms can stop proliferation and osteogenic differentiation of MSCs. KYN also act on AhRs. Activation of AhR connected with ERK pathway can inhibit pre-osteoblasts and osteoblast differentiation and proliferation. KYNA is NMDA-Rs' antagonist. Blocking NMDA-Rs linked to ERK pathway contributes to decreased proliferation of osteoblasts and also decreased RANKL-induced osteclastogenesis. On the other hand QA is agonist of NMDA-Rs. Activation of these receptors can result in stimulation of RANKL-induced osteoclastogenesis. Contrary to this action, QA can stimulate differentiation of MSCs into osteoblast *via* RUNX2-related mechanism. The same effect is exerted by TRP and PA. Mechanisms which are only suggested and need further examination are presented by dotted line.
ADRβ_2, β_2 adrenergic receptor; AhR, aryl hydrocarbon receptor; ATF4, activating transcription factor 4; BDS, brain-derived serotonin; CREB, 3'-5'-cyclic adenosine monophosphate response element binding; CycD1, cyclin D1; DR, dorsal raphe; ERK, extracellular signal-regulated kinase; FOXO1, forkhead box protein O1; GDS, gut-derived serotonin; IFN- γ, interferon γ; KYN, kynurenine; KYNA, kynurenic acid; LRP5, low-density lipoprotein receptor-related protein 5; MSCs, mesenchymal stem cell; NMDA-Rs, N-methyl-D-aspartate receptors; OPG, osteoprotegerin; PA, picolinic acid; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; RUNX2, runt-related transcription factor 2; Tph1, tryptophan hydroxylase 1; Tph2, tryptophan hydroxylase 2; TRP, tryptophan; QA, quinolinic acid.

isoform of the enzyme. Tph-2 is characteristics of neuronal cells and is linked to serotonin synthesis in the brain. On the other hand tryptophan hydroxylase 1 (Tph-1) is responsible for the synthesis of peripheral serotonin (20).

In organisms, serotonin plays a key role in the central nervous system, the gastrointestinal tract and affects processes like hemostasis. There are also works which indicate the importance of serotonin on bone metabolism. Furthermore, the site of serotonin synthesis is significant because of differences of action on bone (21) (*Fig. 3*).

Gut-derived serotonin

Three serotonin receptors are expressed in osteoblasts: 5-HT-R_{1b}, 5-HT-R_{2a} and 5-HT-R_{2b}. Peripheral serotonin, mainly produced in gastrointestinal tract mucosa, can bind to the 5-HT-R_{1b} receptor, making osteoblasts a target for gut-derived serotonin (GDS). This reaction triggers the 5-HT-R_{1b}/PKA/CREB/cyclins signaling cascade. After binding serotonin to 5-HT-R_{1b}, inhibition of cAMP production and phosphorylation of cAMP response element-binding (CREB) mediated by protein kinase A (PKA) is

observed. Effects of this inactivation are evident in decreased expression of cyclin genes and lead to decreased osteoblast proliferation (21, 22) (*Table 1*) (*Fig. 3*).

Knowledge of the exact mechanism of action of GDS is crucial because of the potential in using its arresting properties in osteoporosis treatment. Kode *et al.* provided evidence that gut-derived serotonin influences osteoblasts proliferation through forkhead box protein O1 (FOXO1) (23). The transcription factor FOXO1 regulates cell proliferation in mammals, whereas in *C. elegans* the homolog of FOXO mediates serotonin signaling depending on environment. Experiments on mice allowed the identification of two key FOXO1 complexes, whose activity is specifically regulated by serotonin through the 5-HT-R_{1b} receptor. Of the first FOXO1 complex is connected with CREB and the second is linked to activating transcription factor 4 (ATF4). The mechanism of action of FOXO1 is directly related to levels of circulating serotonin. Under normal levels of GDS, osteoblast proliferation is not disturbed and FOXO1 interacts with CREB and ATF4. When levels of serotonin are high, FOXO1 prefers creating association with ATF4. This condition also counteracts the forming of FOXO1-CREB. All these actions, which are effect of

Table 1. Effects and mechanisms of action of tryptophan, serotonin and melatonin on bone.

Compound	Effect		Mechanism	References
	Bone formation	Bone resorption		
Tryptophan	stimulating the proliferation and differentiation of BMMSCs;		RUNX2-related mechanism*;	18
Gut-derived serotonin		suppressing osteoblast proliferation;	FOXO1-related mechanism (5-HT-R1b/PKA/CREB/cyclins signaling cascade);	21, 22, 32
Brain-derived serotonin	inhibiting proliferation and differentiation of osteoclasts; stimulating osteoblast proliferation;		inhibiting synthesis of RANKL *via* blocking PKA/ATF4 dependent pathway; *via* molecular clock gene cascade;	22, 23
Melatonin	stimulating differentiation of MSCs into osteoblastic cell lineage; protective effect of mesenchymal stem cells; inhibiting proliferation and differentiation of osteoclasts;	suppressing estrogen influence on bone formation, stimulating proliferation and differentiation of osteoclasts *;	RUNX2-related mechanism; antioxidant properties (weakend H_2O_2-induced MSC apoptosis); stimulating osteoprotegerin expression causing inactivation of RANKL; reducing of circulating levels of gonadal estrogens through melatonin down-regulation of the hypothalamic-pituitary reproductive axis*, interfering with the activation of the estrogen receptor*, regulating activity of the aromatases*;	37, 38, 39, 41

*suggested effect or mechanism - needs further examination;
ATF4, activating transcription factor 4; BMMSc, bone marrow mesenchymal stem cells; CREB, 3'-5'-cyclic adenosine monophosphate response element binding; ERK, extracellular signal-regulated kinase; FOXO1, forkhead box protein O1; MMSc, mesenchymal stem cells; RUNX2, runt-related transcription factor 2; PKA, protein kinase A; RANKL, receptor activator of nuclear factor kappa-B ligand.

high levels of GDS, result in suppressed osteoblast proliferation (23) (*Table 1*) (*Fig. 3*).

Brain-derived serotonin

The fact that GDS cannot cross the blood-brain barrier raised questions about the role of brain serotonin in bone metabolism (21, 22). It is now well known that brain serotonin has the opposite effect of GDS on bone mass. GDS inhibits bone formation, but does not affect bone resorption, whereas brain-derived serotonin (BDS) enhances bone formation and decreases bone resorption. Serotonin receptor 5-HT-R_{2c} is expressed in ventromedial hypothalamic neurons. Through this receptor, dorsal raphe BDS signals decrease sympathetic tone by inhibiting the synthesis of epinephrine. The results of decreased sympathetic tone are evident in osteoblasts due to β_2 adrenergic receptors (ADRβ_2), which are expressed on these necessary for bone synthesis cells. Decreased bone resorption is achieved by inhibiting the PKA/ATF4 dependent pathway, which is connected with the synthesis of receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL is known to be a protein involved in bone resorption through osteoclast activity. Data provided by Chabbi-Achengli *et al.* showed that RANKL induced local serotonin synthesis in osteoclast precursors and enhanced the expression of Tph-1 (24). Increased bone formation is observed thanks to the molecular clock gene/cyclinD1 cascade (22). Opposite to GDS, BDS contributes to osteoblast proliferation (*Table 1*) (*Fig. 3*).

Serotonin and leptin

Substances which influence brainstem-derived serotonin can affect bone metabolism. One of these is leptin - a hormone produced in adipocytes. To be more precise, leptin inhibits bone mass accrual. The exact mechanism of the regulation of bone mass by leptin, proposed by Yadaw *et al.*, is based on a double inhibitory loop (25) (*Fig. 3*). First, leptin inhibits synthesis of BDS. Then, as a consequence of the previous action, sympathetic tone is increased. Sympathetic tone has an effect on both osteoblasts and osteoclasts. Leptin acts by binding to Ob-R_b. Ob-R_b receptors, which are expressed in various hypothalamic neurons, like ventromedial hypothalamic nuclei. Ventromedial hypothalamic nuclei are well known sites of BDS production, but are not located in osteoblasts. Osteoblasts express ADRβ2 receptors and the final effect of reduced bone mass results through sympathetic tone and ADRβ_2 receptors. Furthermore, it is proven that inactivation of these receptors in mice results in a high bone mass (25). Likewise, genetic ablation of the leptin gene in mice leads to increased bone mass. It resulted in greater numbers of osteoblasts and a higher bone formation rate (26, 27).

Serotonin and low-density lipoprotein receptor-related protein 5 (LRP5)

Osteoporosis pseudoglioma syndrome (OPPG) is a disorder with specific eye abnormalities and osteoporosis, with an onset in early childhood. This inherited disease is caused by a loss-of-function mutation in LRP5 (28). The opposite type of mutation in LRP5, a gain-of-function, causes high bone mass. The connection between LRP5 and bone metabolism is obvious, but the exact mechanisms are still not well understood. Two models of action currently exist (*Fig. 3*). In the first model, LRP5 functions as a Wnt coreceptor and controls bone mass through canonical Wnt signaling. Data in favor of this model were given by Cui *at al*. (29). The second model is based on inhibition Tph-1 expression, which leads to suppression of serotonin synthesis in the duodenum. Decreased level of GDS results in decreased 5-HT-R_{1b} signaling in osteoblasts, increased CREB expression and function as well as increased CycD1 expression. All these elements contribute to osteoblast proliferation and promote bone mass accrual. This mechanism of action was proven by Yadav *et al.* by using microarray and gene deletion in mice (30, 31). It is also supported by observation that levels of GDS were significantly elevated in OPPG patients (30-32).

Tryptophan hydroxylase 1 (Tph-1) inhibitors

Osteoporosis is a disease whose onset has a clear connection to menopause. It is a prominent problem, especially among postmenopausal women with decreased levels of estrogen. The most popular model research on postmenopausal osteoporosis is the ovariectomized rat model (OVX). Ovariectomy induces disruption of bone remodeling, leading to osteoporosis. Fu *et al.* used this model in experiments where they examined ursolic acid derivative's influence on Tph-1 (33). Results of this experiment revealed that these specific ursolic acid derivatives cure OVX in an anabolic mechanism of action. Given the known fact that Tph-1 is the principal enzyme of GDS biosynthesis, potential Tph-1 inhibitors could become a therapeutic strategy for treating of osteoporosis in the future (33, 34).

Selective serotonin reuptake inhibitors (SSRIs) and bones

The involvement of serotonin in bone metabolism encourages researchers to discover the exact mechanism action of serotonin, focusing on a deeper understanding of the process and making the discovery useful for potential targets for new osteoporosis therapies. Additionally, it gives the impetus to consider serotonin's influence on bone drugs, which are now used in various disorders and interact with the serotonin system. One such group of drugs is selective serotonin reuptake inhibitors (SSRIs). SSRIs are commonly used antidepressants with high affinity for the serotonin transporter (5-HTT). Serotonin transporters are located in osteocytes, osteoblasts and osteoclasts, which raise the question about the potential of SSRIs to increase the risk of bone fracture. In their work, Verdel *et al.* show that the risk of osteoporotic fracture is statistically much higher for antidepressants with high affinity for the 5-HTT. The severity of risk clearly depends on the degree of 5-HTT inhibition (35). Conclusions drawn by Hodge *et al.* are that the use of SSRIs is linked to lower bone mineral density in postmenopausal women and men, as well as a higher risk of fracture (36). An important issue is that, as indicated by Verdel *et al*., antidepressants interact with a lot of neurotransmitter receptors and the participation of other mechanisms cannot be excluded. An example is clomipramine, with its high affinity for the 5-HTT, but its administration is not associated with osteoporotic fractures (35).

MELATONIN

Melatonin fulfills many physiological functions, and it can also affect bone metabolism. Receptors for melatonin MT_1 and MT_2 are expressed on osteoblasts and osteoclasts. Some studies also indicate that melatonin can be produced locally in bone marrow. Described results of melatonin's action on bone are anabolic and antiresorptive effects (*Table 1*) (*Fig. 4*). Studies with human mesenchymal stem cells showed that melatonin increased differentiation of these cells into an osteoblastic cells lineage. This action is associated with augmented expression of RUNX2 and bone morphogenic proteins -2 and -4. Additionally, melatonin can suppress γ peroxisome proliferator-activated

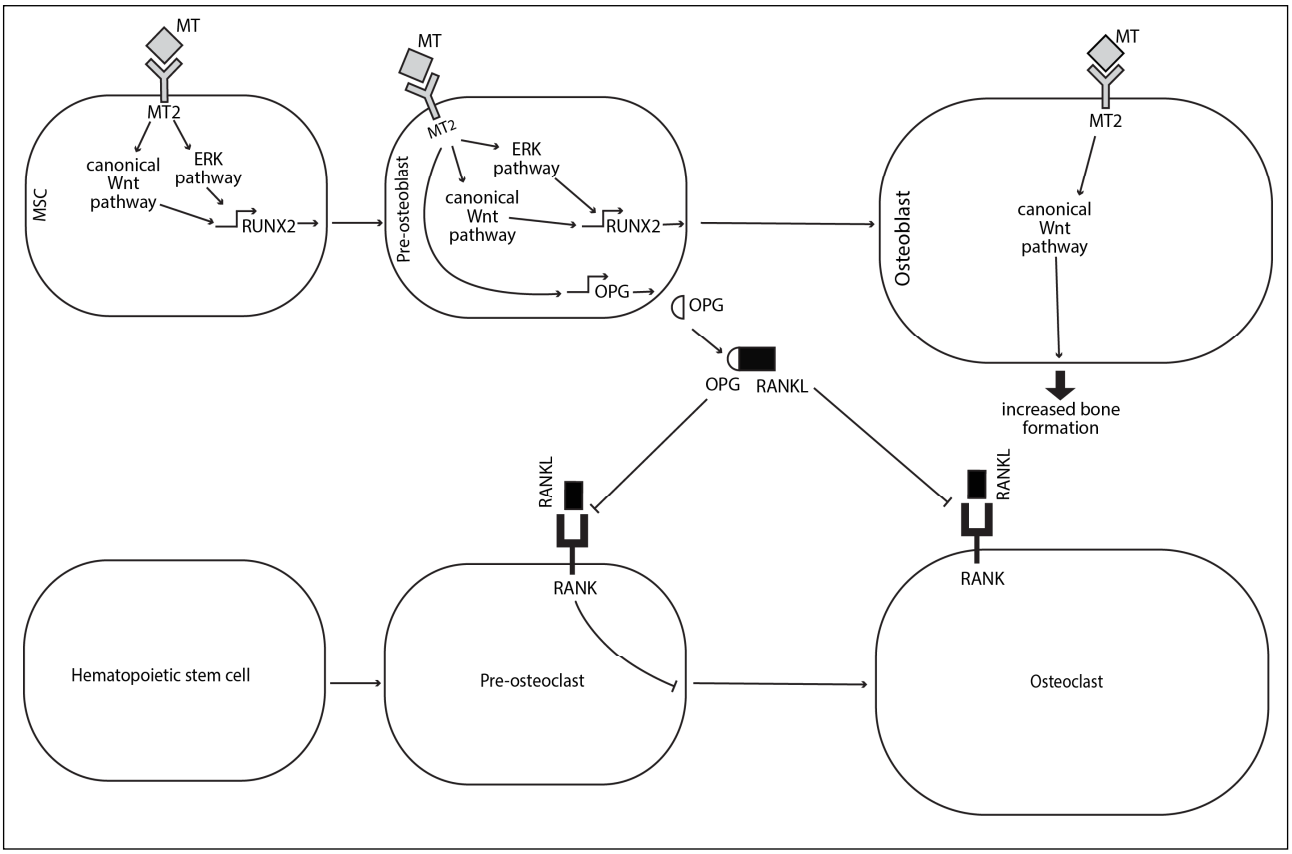

Fig. 4. Influence of melatonin on bone cells. Melatonin exerts anabolic and antiresorptive effects on bone. Through binding to MT_2 receptors on MSCs, melatonin increases differentiation of MSCs into osteoblasts *via* canonical Wnt and ERK pathway. Binding to MT_2 receptors on pre-osteoblasts activates the same pathways and stimulates pre-osteoblasts' differentiation, and increases synthesis of osteoprotegerin, which inhibits differentiation of osteoclasts by prevention of binding RANKL to RANK. MT_2 receptors are expressed also on osteoblasts. Its activation leads to increased differentiation and proliferation of these cells. ERK, extracellular signal-regulated kinase; MSCs, mesenchymal stem cells; MT, melatonin; MT_2, melatonin receptor 2; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; RUNX2, runt-related transcription factor 2.

receptors (PPAR-γ) and reduce oxidative stress (37, 38). Wang *et al.* showed protective effect of melatonin on mesenchymal stem cells (MSCs) (39). Pretreating of this compound significantly weakened H_2O_2-induced MSCs apoptosis. This effect was dependent on dose. It has been also suggested that melatonin reduces the synthesis of RANKL (37-39).

Other evidence suggesting melatonin's impact on bone metabolism is seen in animal and human studies. In pinealectomized animals, bone marker turnover increased and bone mineral density was lessened. Data from studies with melatonin administration to rodents however, showed enhancement in bone mass. In humans, increased bone resorption may be linked to an age-related drop in melatonin levels at night. Moreover, night-time work is connected with a higher risk of osteoporotic fractures (37-39). Additionally, adolescent idiopathic scoliosis is associated with the dysfunction of the melatonin signaling pathway, but the exact etiology is not known. Disturbances in melatonin synthesis can be linked to other disease with bone abnormalities such as Smith-Magenis and Klinefelter syndromes. In the latter, concentration of melatonin is decreased (37). In Smith-Magenis syndrome patients, Elsea *et al.* described inversion of melatonin levels during the day (40). Contrary to the bone-protective effect of melatonin, some findings indicate an antiestrogenic action of melatonin, which may suppress estrogen's influence on bone formation (*Table 1*). These theories however need further research (41). In the Melatonin Osteoporosis Prevention Study by Kotlarczyk *et al.* 18 women received 3 mg of melatonin for 6 months, although results did not show significant changes in bone density (42). Effects of melatonin in treatment of osteopenia are also assessed in randomized control trials Treatment of Osteopenia with Melatonin ended in June 2014. Results are not published yet, but 'Melatonin-Micronutrients for Osteopenia Treatment Study' is still ongoing (38).

KYNURENINE PATHWAY

Tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO)

Enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) play a major role in the first step of the kynurenine pathway (*Fig. 3*). These enzymes convert TRP to N-formylkynurenine, which is non-enzymatically turned into the stable product - kynurenine. TDO and IDO in normal physiological conditions act in parallel. However, in pathological conditions this balance is disturbed, resulting in decreased TDO activity and increased IDO activity (11).

The first step of the kynurenine pathway in the liver is primarily catalyzed by TDO, but this enzyme is also expressed

in central nervous system (11, 41). IDO can be found throughout the body in most cell types. 2007 brought the discovery of a novel IDO-related tryptophan catabolic enzyme - a second isozyme which was named IDO-2. IDO-2 shows similar structural and enzymatic activities to IDO. In contrast to IDO, IDO-2 is less often expressed (43-45).

The induction of IDO, which results in turning TRP into kynurenine, can be initiated by inflammatory factors, like amyloid peptides, lipopolysaccharides, HIV proteins and interferon γ (IFN-γ). IFN-γ is known to be the most potent activator of IDO, which could induce IDO in MSCs. IFN-γ induces gene expression and enzymatic activity of indoleamine 2,3-dioxygenase-1. The findings of Meisel *et al.* show that in MSCs IDO is stimulated by IFN-γ in a dose-dependent manner (46).

Indoleamine 2,3-dioxygenase IDO and interferon γ (IFN–γ)

MSCs are multipotent cells which have the ability to differentiate into a variety of cell types like osteoblasts, chondrocytes and adipocytes. Besides originating from bone marrow, MSCs have been isolated from post-natal and fetal tissues. Due to their ability, MSCs are a promising tool which can be used in regenerative and transplant medicine (10, 12). What is important is that MSCs have potent immunomodulatory functions. MSCs like professional antigen-presenting cells inhibit T-cell response by IDO-mediated tryptophan degradation. These cells inhibit T-cell proliferation by stopping the cell cycle in the G1 phase. Immunomodulatory functions of MSCs also affect other cell function, for example proliferation of NK and B cells, as well as dendritic cell maturation (10, 12, 46). IDO expression by MSCs is not constitutive, but like aforementioned, depends on IFN-γ. To confirm the involvement of IDO in suppressive effects of MSCs on T cell, studies with IDO inhibitors were examined. The result of this was that using IDO inhibitors was linked to the prevention of a reduction in T cell proliferation. In some works TRP replacement was assessed as a possibility of reversion inhibition of T cell proliferation, but it was not effective (46). This fact suggests that products of the kynurenine pathway can be involved in this process.

In regard to bones, Vidal *et al.* showed in their work that the addition of INF-γ accelerated human MSC differentiation into osteoblasts in a dose-dependent manner (12) (*Fig. 3*). The mechanism of action which IFN-γ exerted on bone formation consists of IDO-mediated kynurenine pathway activation. This is proven by two facts. The first is that blocking IDO leads to the inhibition of osteoblastogenesis. The second fact is taken from findings from IDO knockout mice, which showed that these mice exhibited osteopenia with an increased number of osteoclasts and a decreased number of osteoblasts. Anabolic doses of IFN-γ activate IDO and TRP degradation *in vitro* and *in vivo*. These suggest that IDO plays an important role in osteoblastogenesis and bone formation (12). On the other hand, some studies using mice show that IFN-γ, in inflammatory conditions, contributes to enhanced bone degradation *via* T cell receptor activator of nuclear factor κ-B (RANK) signaling. Gao *et al.* proved that in experimental models of postmenopausal osteoporosis, infection induced by bacteria and inflammation preponderated pro-osteoclastogenic properties of IFN-γ (47). Likewise, in humans, treatment of osteopetrosis known as marble bone disease, in which bones become pathologically denser, IFN-γ has been shown to be effective in increasing bone resorption (48). Above and beyond IFN-γ activity, IDO can wield influence on T cells. Impacts of T cell can be achieved by IDO, by inhibiting T cell proliferation or reducing levels of TRP, which results in suppression of T cell activity. IDO can affect regulatory T cells as well. Suppression of these cells is proposed to be one of the mechanisms of bone loss in disorders like bisphosphonate-related osteonecrosis of the jaw-like disease (49).

The kynurenine/tryptophan ratio is an indicator of both inflammation caused by IFN-γ and IDO activity. Another indicator of INF-γ-mediated inflammation is plasma neopterin, which is released from macrophages after IFN-γ stimulation. It is known that low grade chronic systemic inflammation is connected to an increased risk of fractures and bone loss, which generally leads to a higher risk of osteoporosis. The Hordaland Health Study assessed the links between hip fractures and metabolites of the kynurenine pathway, as well as the association between this pathway's metabolites and hip bone mass density. In reference to bone mineral density, findings indicate an inverse association between the kynurenine/tryptophan ratio and bone mineral density only in the oldest populations, and only in male patients (50). This result is in accordance with studies which indicate that IFN-γ enhances bone degradation in inflammatory conditions. As it relates to the risk of hip fractures, there was no link between the kynurenine/tryptophan ratio and the risk of hip fractures (50, 51).

KYNURENINE

Kynurenine is a compound produced in the first step of TRP degradation through the kynurenine pathway. Three compounds are produced directly from kynurenine. Kynurenine aminotransferase converts kynurenine into kynurenic acid (KYNA), kynurenine 3-monoxygenase converts kynurenine to 3-hydroxykynurenine (3-HKYN), and kynureninase converts kynurenine to anthranilic acid (AA) (*Fig. 2*). Studies have linked increasing levels of kynurenine with increasing age (18, 52). Other studies have shown heightened levels of kynurenine in disorders like chronic kidney disease (53) and rheumatoid arthritis (54). Kynurenine, as an oxidation product of TRP, exhibited the ability to stop BMMSCs proliferation and osteogenic differentiation (*Table 2*) (*Fig. 3*). Increasing levels of pathogenic kynurenine due to aging and the inhibition of anabolic signals leads to the development of osteoporosis (18).

Kynurenine's other attributes can indicate its role in metabolic bone disease. Kynurenine can act on the aryl hydrocarbon receptor (AhR) (55, 56) (*Fig. 3*). AhR is the ligand-activated transcription factor which belongs to the transcription factor superfamily, and plays a key role in the modulation of the immune system. Kynurenine, which is endogenous AhR ligand, can augment IgE-mediated mast cell activation *via* AhR. These effects are confirmed by data from AhR deficient BMMCs, which show that potentiation of IgE-mediated mast cell activation was not observed in these cell, and by data from studies with AhR antagonist which indicate on inhibiting discussed effects by AhR antagonist (57). AhR may impact regulatory T cells and IL-17 producing T helper cell equilibrium, because generation of these cells is controlled by AhR (58). As was pointed earlier, suppression of regulatory T cells is the putative cause of bone loss in bisphosphonate-associated osteonecrosis of the jaw (49).

Other described AhR functions are connected with normal bone development. Herlin *et al.* used AhR knockout mice, in which alterations in cortical and trabecular bone tissue were observed (59). In Yu *et al.* findings mice with AhR deletion in osteoclasts showed an increase in bone mass and decreased bone resorption. *In vitro*, murine cells stopped differentiation and weakened osteoclastogenesis were noticed (60). Another study with the collagen-induced arthritis model showed that AhR can inhibit osteoblast proliferation and differentiation *via* the

extracellular receptor-activated kinase (ERK) signaling pathway, which can cause bone erosion in arthritis. AhR-dependent activation of this signaling pathway can inhibit pre-osteoblast proliferation and differentiation too (61). Additionally, AhR ligands can affect bones. Dioxins, which can be found among environmental pollutants, have a high affinity to AhR and can induce osteopenia (56, 59, 60). Exposure to dioxins can result in decreased bone mineral density and strength, as well as altered geometry in bone tissue (59, 60, 62). Bones exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) were more vulnerable to micro-cracking (59). The effects of inhibition of dioxin on bone formation were achieved by using AhR antagonists (59, 63). All these findings suggest that treatment with AhR antagonists and inhibition of AhR in the osteoclast lineage can bring potential benefits in treatment of bone metabolism diseases. Further research linking kynurenine, AhR and bones is required.

KYNURENIC ACID

Kynurenic acid (KYNA), a compound which is produced from kynurenine with kynurenine aminotransferase, is able to block N-methyl-D-aspartate (NMDA) receptors (52, 55). It has been shown that KYNA, at low concentrations, can impact the glycine modulatory site of NMDA-Rs. When the concentration is increased however, KYNA affects the glutamate site of NMDA-Rs (49). Besides this, KYNA can act on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and is also known as a non-competitive antagonist of α-7-nicotinic-acetylocholine receptors (α7NR). Additionally, another target for KYNA is the orphan G-protein-coupled receptor GPR35, which can be activated by the discussed compound (52, 55). Studies showed that in patients with inflammatory bowel diseases, the number of GPR35 is increased, which can be linked to

Table 2. Effects and mechanism of action of kynurenine derivatives on bone.

Compound	Effect		Mechanism	References
	Bone formation	Bone resorption		
Kynurenine		blocking BMMSC proliferation and osteogenic differentiation;	RUNX2-related mechanism*; *via* ERK-pathway*;	18
Kynurenic acid		blocking differentiation of osteoblasts and RANKL-induced osteclastogenesis*;	antagonist of NMDA-Rs; *via* ERK-pathway*;	52, 55, 64
Anthranilic acid	antioxidant properties;		inhibiting 3-hydroxyanthranilic acid oxidase which converts 3-HAA into QA with pro-oxidant properties;	65, 66
3-hydroxy-anthranilic acid	antioxidant properties;	pro-oxidant properties;	–	52, 65, 66
3-hydroxy-kynurenine		reducing viability osteoblast-like cells;	–	69
Picolinic acid	osteogenic potential - recovering the capacity of BMMSC obtained from IDO1−/− mice to differentiate and inducing osteoblasts differentiation;		RUNX2-related mechanism*;	12
Quinolinic acid	osteogenic potential - recovering the capacity of BMMSC obtained from IDO1−/− mice to differentiate;	enhancing RANKL-induced osteclastogenesis*;	RUNX2-related mechanism*; agonist of NMDA-Rs on osteoclast*;	12, 52, 74

*suggested effect or mechanism, needs further examination; - mechanism is not described;
3-HAA, 3-hydroxyanthranilic acid; BMMSc, bone marrow mesenchymal stem cells; ERK, extracellular signal-regulated kinase; IDO1, tryptophan 2,3-dioxygenase; NMDA-Rs, N-methyl-D-aspartate receptors; RANKL, receptor activator of nuclear factor kappa-B ligand; RUNX2, runt-related transcription factor 2; QA, quinolinic acid.

augmented levels of KYNA (55). Levels of KYNA can be very different in various diseases. For example, in chronic renal failure, this level is elevated in hepatic and renal tissue, as well as in the lungs, intestine, and spleen (53). Concentration of KYNA, considered as an endogenous neuroprotector and compound having anti-epileptic actions (52, 55), was decreased in the brain in Huntington's disease. In contrast, patients with schizophrenia were diagnosed with increased levels of KYNA in cerebrospinal fluid (52). Elevation of these levels was also noticed with progressing age. (55).

Apart from a reduction of fibroblastic growth factor-1 and an impact on different factors (52, 55), KYNA is an antagonist at glutamate receptors, which was mentioned above (54). Glutamate receptors are expressed on osteoclast cells, which suggest that KYNA can influence bone remodeling. Antagonists of NMDA-Rs can inhibit differentiation of osteoblasts and RANKL-induced osteoclastogenesis (*Table 2*) (*Fig. 3*). There is also evidence that activation of NMDA-Rs is connected with the ERK signaling pathway (64). Forrest *et al.* measured levels of KYNA in rheumatoid arthritis and osteoporosis patients, but they did not notice any changes (54, 65). On the other hand, data from Hordaland Health Studies present a connection between KYNA and bone mass density (50).

ANTHRANILIC ACID AND 3-HYDROXYANTHRANILIC ACID

Another compound that is produced from kynurenine, in this case with kynureninase, is anthranilic acid (AA). AA can be converted into 5-hydroxyanthranilic acid or 3-hydroxyanthranilic acid (3-HAA). The latter compound can also be produced by the conversion of 3-HKYN (*Fig. 3*). Modified levels of AA and 3-HAA can be found in various disorders like neurodegenerative and psychiatric disorders, as well as in coronary heart disease, stroke or osteoporosis.

In patients with osteoporosis, the concentration of 3-HAA was decreased, but levels of AA were increased when compared with controls. Darlington *et al.* additionally assessed the 3-hydroxyanthranilic acid: anthranilic acid ratio (3-HAA:AA), which was reversed in patients with osteoporosis (65, 66). In regard to rheumatoid arthritis, patients suffering from this disease showed decreased levels of 3-HAA as well (54, 65-67). Besides this, the Igari *et al.* study presents data of heightened levels of AA in synovial fluid from patients with rheumatoid arthritis when compared to patients with osteoarthritis (68). In addition to changes in 3-HAA and AA levels, high oxidative stress levels were also noticed. As is known, 3-HAA exhibits pro-oxidant and antioxidant activity, which depends on local redox conditions (52, 55, 65) (*Table 2*). Darlington *et al.* (66) suggests that decreased concentrations of 3-HAA can be one of causes of osteoporosis due to insufficient antioxidant protection. Arguments in favor of this were observed in patients with osteoporosis, in which augmented markers of oxidative stress were noticed (55, 65, 66). 3-HAA can be converted into picolinic acid (PA) and quinolinic acid (QA), which is a neurotoxin with pro-oxidant properties. AA can reduce this conversion by inhibiting 3-hydroxyanthranilic acid oxidase (3-HAO) (*Table 2*). There is a possibility that this action serves to reduce cell toxicity (65, 66). The same studies, which include described changes in patients with osteoporosis, showed the influence of the treatment on levels of 3-HAA and AA. After an effective two years of therapy with raloxifene or disodium etidronate, concentrations of 3-HAA and AA were all consistent with control levels (65). These data, along with findings from the Hordaland Health Study, show that 3-HAA is positively connected with bone mineral density in women and that AA high levels are linked to a higher risk of hip fracture (50, 51). This suggests that antioxidant activity of 3-HAA can be one of the factors exerting influence on bone metabolic disease.

3-HYDROXYKYNURENINE

3-hydroxykynurenine (3-HKYN) is formed from kynurenine with kynurenine-3-monooxygenase. In the next steps, this compound can be converted into aforementioned 3-hydroxyanthranilic acid or xanthurenic acid (*Fig. 2*). 3-HKYN can produce free radical species, which may result in initiation of apoptosis (54, 55, 69). 3-HKYN is also known as a neuroactive compound, revealing cellular toxicity (52, 55). Fatokun *et al.* showed that 3-HKYN reduced the viability of osteoblast-like cells (69) (*Table 2*).

Hordaland Health Study revealed that 3-HKYN is connected with a higher risk of hip fracture. Additionally, levels of 3-HKYN were more augmented among women than men in the middle-age group of examined patients (50, 51). In another study, 3-HKYN had a positive correlation to inflammatory markers (70). The latter connection of 3-HKYN with a higher risk of hip fracture tie 3-HKYN to bone metabolic disease. Moreover, levels of 3-HKYN in patients with rheumatoid arthritis were decreased. Forrest *et al.* suggest that the reduction of 3-HKYN concentration can be a consequence of the conversion into xanthurenic acid by the kynurenine aminotransferase enzyme (54).

XANTHURENIC ACID

Xanthurenic acid is a compound for which Malina *et al.* proved ability to induce cell apoptosis connected with nuclear DNA cleavage, cytochrome C release and activation of caspase -3, -8 and -9 (71). In patients with rheumatoid arthritis, concentration of this compound was augmented. In patients with osteoporosis however, the Hordaland Health Study positively linked xanthurenic acid concentration to bone mineral density in all sex and age groups (50, 54). These contradictory data indicate that further investigation about the role of xanthurenic acid in bone metabolism is necessary.

PICOLINIC ACID

Picolinic acid (PA), an isomer of nicotinic acid, is produced from 3-hydroxyanthranilic acid trough 2-amino-3-carboxymuconate-semialdehyd (*Fig. 2*). It is considered as an endogenous neuroprotectant with the ability of chelating zinc or iron for example, and blocking neurotoxicity caused by QA. Some studies showed antiviral and apoptotic activity of this compound on HIV-1 and HSV-2 (12, 52).

Vidal *et al.* assessed activity of PA in relation to bones. They treated human MSCs with three different doses of PA and observed that it exerted strong and dose-dependent osteogenic effects *in vitro*. Additionally, this effect was connected with augmented levels of expression of the osteogenic genes RUNX2 and osteocalcin in cells (*Table 2*) (*Fig. 3*). Data from testing the effect of PA on *ex vivo* cultures of bone marrow stromal cells from IDO KO mice showed that picolinic acid caused augmentation in a number of colony-forming units-osteoblasts. What is important, this effect exceeded the osteogenic upshot of osteoblastogenesis induction medium on bone marrow stromal cells from wild type mice. These findings, along with the fact that high doses of PA are well tolerated, indicate that PA can be a potential bone anabolic used in osteoporosis treatment (52).

QUINOLINIC ACID

Quinolinic acid (QA), previously mentioned in the context of its neurotoxicity, is another compound produced *via* the kynurenine pathway. It selectively activates NMDA-Rs (*Table 2*). This effect, plus the ability to generate ROS, is considered a foundation of neurotoxic action. Besides QA having immunomodulatory activity, it has shown that its concentration increased during immune response. Moreover, QA is associated with neurodegenerative disorders, like Alzheimer's disease, Huntington's disease and dementia due to HIV (52, 55, 72, 73).

In view of bone, QA as NMDA-Rs subtype of glutamate receptors agonist has possible impact on bone metabolism, because of proof about the occurrence of glutamate receptors on osteoclasts and osteoblasts (74). In a study by Vidal *et al.* osteogenic potential was noticed after treating bone marrow stromal cells with QA (*Table 2*) (*Fig. 3*). The observed effect was weaker however than obtained after PA treatment (12).

CONCLUSION

In recent years, knowledge about serotonin's action has increased. However, further examinations are still required to define its precise mechanism of action and to evaluate the potential of inhibiting Tph-1. We believe that blocking this principal enzyme of GDS synthesis is the right course to intensify research (33). As it was mentioned before, serotonin exerts two-sided effect on bone (21), so the next important issue is the safety of drugs, which interact with the serotonin system and are currently used in treatment of various disorders (35, 36).

From all TRP metabolites - melatonin is the most widely used in clinical practice, as it relates to bones. Despite the fact that data from animal studies are promising (37-39), results from clinical trials will reveal its real usability in the treatment of bone disease (38).

Kynurenine derivatives and its influence on bones are still the least well known from all the TRP metabolic products. Knowledge about mechanisms underlying its action on bone and bone cells is very limited and its current level only gives an indication of its multi-faceted effect. From investigations of the kynurenine pathway, we find extraordinarily interesting areas regarding AhR receptors and its agonist - KYN, as well as PA - a compound which is well tolerated even at high doses (52) and showed osteogenic potential (12).

The discovery of the influence of tryptophan's metabolic products on the process of bone remodeling sets new targets for pharmacological studies. At a time of dynamically developing research, special attention should be paid to discover the exact mechanism of action of melatonin, serotonin and currently not well known kynurenines in the context of bone, bearing in mind increasing life expectancy and other factors contributing to development of osteoporosis. Currently, insufficient therapy of this disease demonstrates the need for a continuation and expansion of studies in this field, which may lead to finding a new effective therapy and more importantly - a better quality of life for patients in the future.

Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranilic acid oxidase; 3-HKYN, 3-hydroxykynurenine; 5-HT-R, serotonin receptor; 5-HTT, serotonin transporter; AA, anthranilic acid; ADRβ_2, β_2 adrenergic receptor; AhR, aryl hydrocarbon receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ATF4, activating transcription factor 4; BDS, brain-derived serotonin; BMMSCs, bone marrow mesenchymal stem cells; cAMP, 3'-5'-cyclic adenosine monophosphate; CREB, 3'-5'-cyclic adenosine monophosphate response element binding; CycD_1, cyclin D_1; ERK, extracellular signal-regulated kinase; FOXO1, forkhead box protein O1; GDS, gut-derived serotonin; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus type 2; IDO, indoleamine 2, 3-dioxygenase; IFN- γ, interferon γ; KO, knockout; KYNA, kynurenic acid; LRP5, low-density lipoprotein receptor-related protein 5; MIO, male idiopathic osteoporosis; MSCs, mesenchymal stem cells; MT_1, melatonin receptor 1; MT_2, melatonin receptor 2; NAD, nicotinamide adenine dinucleotide; NMDA, N-methyl-D-aspartate; NMDA-Rs, N-methyl-D-aspartate receptors; OPPG, osteoporosis pseudoglioma syndrome; OVX, ovariectomized rat model; PA, picolinic acid; PKA, protein kinase A; PPAR-γ, γ peroxisome proliferator-activated receptors; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; ROS, reactive oxygen species; RUNX2, runt-related transcription factor 2; SSRIs, selective serotonin reuptake inhibitors; TCDD, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin; TDO, tryptophan 2, 3-dioxygenase; Tph, tryptophan hydroxylase; TRP, tryptophan; QA, quinolinic acid; α7NR, α-7-nicotinic-acetylocholine receptors.

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