Regulation of Bone Metabolism by Serotonin

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Introduction

The bone is a highly dynamic tissue that is influenced by many intrinsic and extrinsic signaling factors capable of modulating its growth and turnover. The balance between new bone formation by osteoblasts and resorption of calcified bone by osteoclasts is responsible for accurate bone modeling and remodeling. Impairment in this equilibrium can lead to skeletal disorders involving bone loss, such as osteoporosis, or the contrasting high bone mass syndromes that result from genetic mutations [1]. A variety of hormones, growth factors, and cytokines regulate the coordinated activity of osteoblasts and osteoclasts. Recently, serotonin (5-hydroxytryptamine; 5-HT) is becoming appreciated as one of the key players in bone tissue dynamic, and the mechanisms by which it acts are still being revealed [2]. Significantly, 5-HT can exert divergent effects on bone density, both through actions in the brain and through actions on cells within the bone [3].

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Serotonin as a Signaling Molecule in the Brain and Periphery

While serotonin is best known for the roles it plays as a neurotransmitter in the central nervous system (CNS), it was first discovered in the periphery during searches for signaling molecules that could contract smooth muscle. In 1937, the Italian physiologist Vittorio Erspamer isolated an indole molecule, highly abundant in the gastrointestinal (GI) tract of various species, which caused muscle contraction [4]. He named this compound "enteramine" and described it as the main secretory molecule of enterochromaffin (EC) cells. Nearly a decade later, Maurice Rapport in collaboration with Arda Green and Irvine Page (1948) identified a compound in bovine serum that caused blood vessel contraction, and they named it "serotonin" [5, 6]. Structural analysis of enteramine and serotonin proved that enteramine and serotonin were the same molecule: 5-hydrotryptamine (5-HT) [7]. It was Betty Twarog, who in 1953 demonstrated the presence of serotonin in the mammalian brain and suggested a role for the molecule as a neurotransmitter rather than a hormone [8]. Since the blood-brain barrier is quite impermeable to serotonin, serotonin pools in the CNS vs the periphery are considered as two distinct pools that are independently regulated.

Despite its widespread influences, serotonin is produced by relatively few cells in the body. Within the CNS, serotonin is synthesized by clusters of neurons, designated B1-B9, that are restricted to the brainstem raphe nuclei. These neurons, which use tryptophan hydroxylase 2 (TPH2) as their rate-limiting enzyme for serotonin synthesis, extend ascending and descending axonal projections that reach most regions of the CNS and mediate a wide variety of functions. The axon terminals of these neurons express the serotonin-selective reuptake transporter (SERT) in their membranes, so once serotonin is released from the nerve terminals and mediates its actions on nearby receptors, it is removed from the synaptic cleft. Within serotonergic nerve terminals, serotonin is deaminated by monoamine oxidase A (MAO-A) and converted to 5-hydroxyindole acetic acid (5-HIAA) by aldehyde dehydrogenase [9].

In the periphery, the major site of serotonin synthesis is the EC cells of the intestinal mucosa, which are a form of enteroendocrine cells, and in fact EC cells are the source of the vast majority of the serotonin in the body [10]. EC cells use TPH1 to synthesize serotonin, as do all other peripheral serotonin-synthesizing cells, with the exception of enteric serotonergic neurons, which like CNS neurons utilize TPH2. After EC cells release serotonin in response to chemical and/or mechanical stimuli, it acts as a paracrine signaling molecule on nearby nerve fibers, epithelial cells, and immune cells that express serotonin receptors, and it is then taken up by epithelial cells, all of which express SERT. The serotonin that is not transported into epithelial cells moves into the blood stream, where it is transported into platelets, which also express SERT. Until recently, it was presumed that intestinal EC cells represented the sole source of peripheral serotonin because 5-HIAA levels in the urine are undetectable within 24–48 h after removal of the entire GI tract from experimental animals [11]. However, the finding that non-neuronal serotonin is

synthesized by TPH1 has led to the discovery of local sources of peripheral serotonin synthesis, including adipocytes [12], pancreatic islet β-cells [13, 14], and cells within the bone [15].

Serotonin and Bone Remodeling in Health

Brain-Derived Serotonin

Serotonin in the CNS has a positive effect on bone growth, since young TPH2-/- mice (12 weeks and younger) have low bone mass with decreased bone formation [16]. The osteogenic actions of serotonin in the CNS involve the inhibition of inhibitory inputs in the following sequence of events (Fig. 1a): (1) serotonergic raphe neurons provide excitatory input to neurons in the ventromedial hypothalamus; (2) hypothalamic neurons provide inhibitory input to sympathetic preganglionic neurons; and (3) the decrease in sympathetic outflow releases the bone from the resorption influence of β2 adrenoreceptor activation. Therefore, the CNS serotonergic system favors bone accrual. In older TPH2-/- mice (21–83 weeks), moderately elevated trabecular bone in the vertebrae has been reported in both sexes, and females have decreased femur cortical bone [17].

Central serotonin regulates bone mass accrual through stimulation of 5-HT_{2C} receptors on hypothalamic neurons, while appetite, which is also influenced by leptin acting on serotonergic neurons, is controlled by activation of 5-HT_{1A} and $_{2B}$ receptors in the arcuate nucleus [16, 18]. Activation of 5-HT_{2C} receptors leads to decrease sympathetic tone that is activated by calmodulin kinase (CaMK)-dependent signaling cascade. Decreased sympathetic tone releases bone cells from the impact of β_2 adrenoreceptor activation, which can promote bone growth through two opposite actions: by promoting the proliferation of osteoblasts and by inhibiting the proliferation and differentiation of osteoclasts.

The CNS serotonin bone pathway is negatively regulated by leptin arising from adipocytes. Both Ob/ob and db/db mouse models, which lack leptin or the leptin receptor, respectively, have increased bone mass, and this bone phenotype can be corrected by specific inactivation of central serotonin signaling [2, 16, 19]. Furthermore, deletion of the leptin receptor on brainstem neurons, which would release these neurons from the inhibitory influence of leptin, mimics the elevated bone mass phenotype seen in the Ob/ob and db/db mouse models. Leptin inhibits serotonergic raphe neurons both by reducing their serotonin synthesis and their level of excitability, which, in turn, results in decreased serotonergic input to the hypothalamic nuclei. It has been reported that leptin regulation of appetite does not involved CNS serotonin neurons, and relevant to this discussion, leptin receptors were only found on non-serotonin neurons in the raphe nuclei [20].
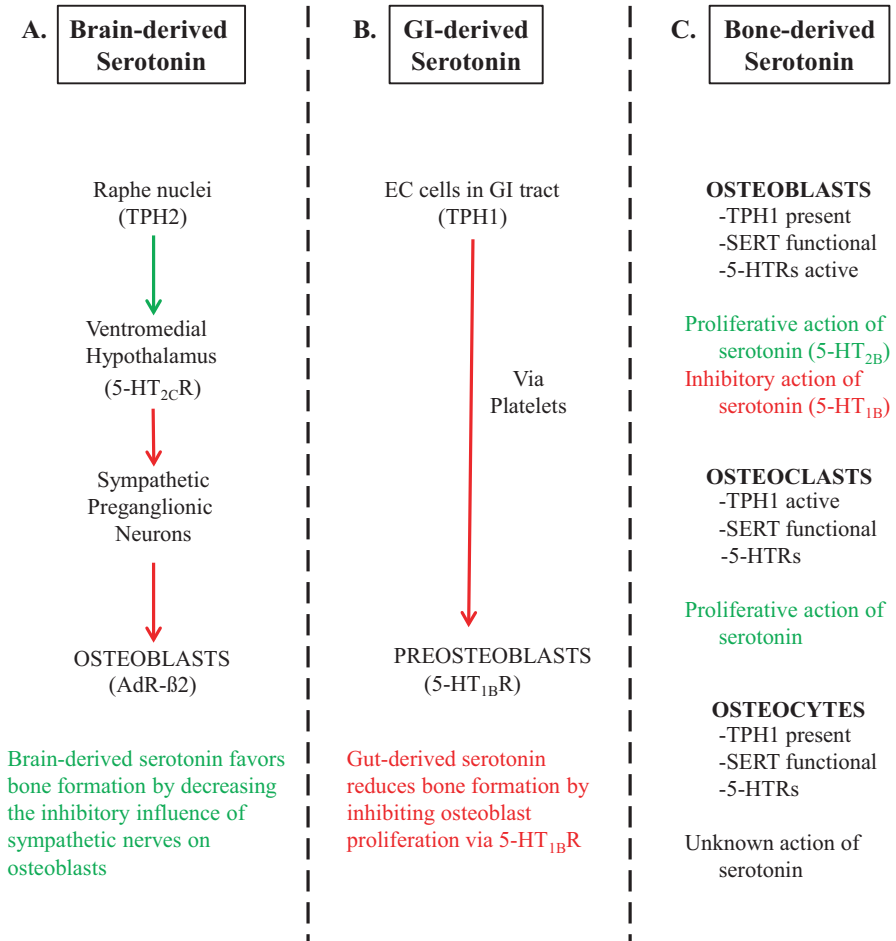
Fig. 1 Summary of the action of serotonin, from different sources, on bone cells. (**a**) Brain-derived serotonin synthesized by tryptophan hydroxylase 2 (TPH2) in raphe nuclei decrease the inhibitory action of the sympathetic neurons by acting on 5-HT_{2c} receptors on ventromedial hypothalamic neurons leading to increased bone formation. (**b**) GI-derived serotonin, synthesized in enterochromaffin (EC) cells by tryptophan hydroxylase 1 (TPH1), enters the blood stream where it accumulates in serotonin-specific reuptake transport (SERT)-expressing platelets. Circulating serotonin inhibits osteoblasts proliferation by its binding on 5-HT_{1B} receptors expressed by pre-osteoblasts. (**c**) Bone-derived serotonin. Bone cells express TPH1, SERT, and 5-HTRs. While serotonin can potentially either promote or inhibit osteoblast proliferation, depending on the receptor that is activated, it has mainly a proliferative action on osteoclasts. Highlights in red are associated with inhibitory pathways, while green highlights represent activation pathways

Therefore, serotonin signaling in the brain can have a positive impact on bone accrual, and this action may be suppressed by the effects of leptin on serotonergic neurons in the raphe nuclei.

Gut-Derived Serotonin

Interest in the role played by gut-derived serotonin in the regulation of bone metabolism developed when a link was identified between a member of the low-density lipoprotein receptor (Lrp) family, Lrp5, and the level of TPH1 expression in the gut [21]. Lineage studies have linked mutations of the Lrp5 gene with changes in bone formation. Loss-of-function Lrp5 mutations are associated with osteoporosis-pseudoglioma syndrome (OPPG), while gain-of-function changes lead to high bone mass (HBM) syndrome [22, 23]. Lrp5 acts as a co-receptor in the canonical Wnt signaling pathway modulating signal transduction in osteoblasts. Using conditional Lrp5 knockout mice, Yadav and Karsenty described bone abnormalities that were different than those seen by inactivation of Wnt signaling in osteoblasts or in osteocytes [21], suggesting that the Lrp5 that influences bone density might be at another site. As in OPPG and HBM patients, Lrp5 null mice present no bone abnormalities at birth, and postnatal changes observed in these mice are not reproduced by blocking the Wnt signaling after birth [3, 22–24]. Transcriptome analysis of Lrp5-/- mice revealed that one of the most dramatic changes was an increase in TPH1 expression, which, as described above, is used by intestinal EC cells to synthesize serotonin [21]. These mice also have increased levels of circulating serotonin and decreased bone density. Mice expressing a gain-of-function mutation of the Lrp5 gene have decreased TPH1 gene expression and lower levels of serotonin in blood. In both Lrp5-/- and TPH1-/- mice, phenotypes were restricted to osteoblast formation, with changes only in the expression of cyclin D1, D2, and E1, all of which are related to cell proliferation as opposed to osteoblast or osteoclast differentiation [21].

The action of serotonin on osteoblast proliferation has been linked to the expression of 5-HT$_{1B}$ receptors by pre-osteoblasts [21]. Binding of serotonin to the 5-HT$_{1B}$ receptor affects the cAMP response element-binding protein (CREB) regulation of osteoblast proliferation through transcription factor FOXO1. The balanced interaction of FOXO1 with transcription factor CREB and activating transcription factor 4 (ATF4) promotes normal proliferation of osteoblast. Elevated circulating serotonin levels are associated with a decrease in the association of FOXO1 with CREB, and this leads to inhibition of bone formation [25].

The concept that gut-derived serotonin can decrease the osteoblast to osteoclast ratio and suppress bone growth has led to the theory that some forms of osteoporosis might be alleviated by downregulating enteric serotonin levels using inhibitors of TPH1. Mouse and rat models of osteoporosis have been studied using LP533401, a TPH1 and TPH2 inhibitor that is not well absorbed and does not readily cross the blood-brain barrier; therefore, its actions are thought to be limited primarily to EC

cells [26]. Daily treatment with this antagonist leads to decreased circulating serotonin levels and a significant elevation in bone formation in ovariectomized mice [27].

It is important to note that a study by another group failed to detect evidence for a role for GI-specific Lrp5 action in bone metabolism. Cui and colleagues were unable to detect bone defects when they inactivate Lrp5 gene specifically in the GI tract [28]. They instead proposed that bone-specific Lrp5 is responsible for changes of function in the osteocyte population through the canonical Wnt pathway [28]. In addition, they were unable to reproduce the anabolic effect of TPH1 inhibitors on bone mass.

This discrepancy has led to an unresolved debate in the field about the importance of gut-derived serotonin as it relates to bone density [29–33], but as proposed, serotonin released from EC cells and entering the blood stream has a negative impact on bone formation by inhibiting osteoblast formation (Fig. 1b). Some differences in the studies most likely arose due to differences in mouse models (strain, age, and sex of the mice), as well as methods and assays that were employed by the different research teams. In the view of the recent data, both mechanisms (1-direct regulation of Lrp5 on osteoblast through a canonical Wnt pathway and 2-indirect paracrine regulation via increase circulatory serotonin and action on pre-osteoblast 5-HT_{1B}) appear to be plausible, and potentially complementary, physiological processes. That said, the results of Yadav's studies raise an additional important issue regarding circulated serotonin: for increased circulatory serotonin to affect bone formation, it would need to be released near its targets, most likely in a regulated manner (pre-osteoblasts or other 5-HTR-bearing cells). Because of its effect on smooth muscle tone, serotonin is efficiently stored in platelets, leaving negligible levels of free serotonin in the serum. At this point little is known about how such targeted release occurs, so more studies of the dynamics of platelet storage and release are needed.

Bone-Derived 5-HT

In addition to the osteogenic actions of serotonin originating in the brain and the gut, another source of serotonin was recently added to the equation: serotonin synthesized within the bone itself (Fig. 1c). Serotonin is synthesized by osteoclast precursors in the presence of NF-KB ligand (RANKL), and downregulation of TPH1 expression by pre-osteoclasts leads to decreased bone resorption and, therefore, increased density. Using mice in which TPH1 is constitutively inactivated, Chabbi-Achengli and colleagues noted that osteoclasts locally produced serotonin that promotes bone formation by increasing the activity of osteoblasts while decreasing the production of osteoclasts [15]. Adult TPH1-/- mice have normal bone density, but elevated bone density was reported at earlier ages, indicating that compensatory mechanisms may correct for altered serotonin signaling.

Bone Expression of SERT and 5-HT Receptors

In addition to expressing TPH1, osteoblasts, osteoclasts, and osteocytes all express functional serotonin transporters [34–37]. As described above, SERT is an important player in serotonin signaling, as it is required for the termination of serotonin signaling. The presence of SERT on bone cells can serve to quickly remove serotonin from the interstitial space. This is supported by knowledge of the actions of serotonin-selective reuptake inhibitors (SSRIs) in the brain, which not only increase serotonin availability and activation of receptors at sites of release but can also lead to longer-term changes such as receptor desensitization and altered levels of receptor expression [38, 39].

A number of 5-HT receptors are also expressed on primary bone cells, including 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2B}$ receptors [34, 37, 40–42]. The inhibitory action of GI-derived serotonin on 5-HT$_{1B}$ receptor, expressed on pre-osteoblast, has been previously described by Yadav and collaborators [21] (Fig. 1b). It is also known that mice lacking 5-HT$_{2B}$ receptor exhibit osteopenia due to impaired bone formation. The absence of the 5-HT$_{2B}$ receptor causes impaired osteoblast proliferation, recruitment, and matrix mineralization involving nuclear PPAR receptors and prostacyclin [43, 44]. Inhibition of 5-HT$_{2A}$ receptors in mice reduces bone mass by decreasing osteoblasts differentiation [45]. While 5-HT$_{1A}$ receptors are found on osteoblasts and osteoclasts, the physiological role of this receptor in vivo is still unclear.

The 5-HT$_6$ receptor is also highly expressed in the bone [46]. Expression of the 5-HT$_6$ receptor is increased during bone remodeling and osteoblast differentiation. In primary cultures of osteoblasts, activation of 5-HT$_6$ inhibits alkaline phosphatase activity and bone mineralization, while in vivo activation of 5-HT$_6$ inhibited bone regeneration and delayed bone development. Thus, serotonin receptor activity contributes to major anabolic functions of the osteoblast for bone formation.

Nonconventional Action of Serotonin in Periphery

In addition to acting conventionally through the activation of receptors expressed on cytoplasmic membranes, serotonin has been shown in a number of systems to mediate its actions via a mechanism called serotonylation. Serotonylation is a receptor-independent process in which serotonin activates intracellular processes. Cui and Kaartinen have reported that serotonylation occurs in the bone [47]. They demonstrated that serotonin can be incorporated into plasma fibronectin by transglutaminase-mediated serotonylation and altered its function. Serotonin interferes with the role pFN in extracellular matrix assembly in osteoblasts and might lead to weaker bones.

Serotonin and Bone Remodeling in Disease

One of the first indications that serotonin plays an important role in bone metabolism came from multiple reports of increased incidence of bone fractures and osteoporosis in patients taking SSRIs to treat depression [48, 49]. These compounds increase serotonin availability by inhibiting SERT, and in the case of serotonin secreted from the gut, their use can lead to increased amounts of serotonin entering the circulation. Many studies have also linked depression to osteoporosis, with more severe depression correlating with higher decreased in bone mineral density. Besides the use of SSRIs, there are a number of confounding factors that can lead to the bone loss observed in depressive patients. Behavioral factors (increased cigarette or alcohol use), biological factors (increased cortisol level and inflammation), and increased incidence of Crohn's disease and diabetes, which are observed in this population, can influence serotonin signaling [50].

The need for a better understanding of the effect of serotonin on bone growth is underscored by the increased use of SSRIs to treat depression, including treatment of adolescents with growing bones and of pregnant women. SSRIs used during pregnancy can cross the maternal-fetal blood barrier leading to potential modulation of fetal serotonin. While some studies have reported increased risks of cleft palates, recent studies have shown normal bone growth during pregnancy although newborns had smaller head circumference and height [51]. Decreased growth has also been observed when youths are prescribed SSRIs to treat depression [52].

The observation that both wild-type mice treated with multiple doses of SSRIs and mice lacking the SERT gene have reduced bone growth supported a direct link between serotonin signaling and bone dynamic [53]. Similar results were obtained using perinatal treatment with tranylcypromine (TCP), a monoamine oxidase (MAO) inhibitor in rats, leading to persistent changes in serotonin availability [54]. Perinatal exposure to TCP leads to decreased TPH1 expression in the peripheral compartment and increased bone volume and trabecular number.

These studies as well as the study of a mouse model of depression [55, 56] suggest that peripheral and central serotonin compartments have different mechanisms to react to 5-HT imbalances and suggest a predominant role for gut-derived serotonin in the regulation of bone maintenance under these conditions. While SSRIs increase 5-HT levels and alleviate both depression and unbalanced sympathetic tone, it has deleterious effects on bone metabolism. Of note is the fact that the negative effects of increased serotonin in the periphery appear to outweigh the bone accrual effects that would be expected to result from the enhanced serotonin signaling in the brain.

Table 1 Summary of serotonin signaling-related proteins expressed in bone.

Model	Net effect on bone metabolism	References
TPH1-/-	Increased osteoblast formation	Yadav et al. [21]
TPH1-/-	No effect on bone metabolism	Cui et al. [28]
TPH1-/-	Decreased bone resorption	Chabbi-Achengli et al. [15]
TPH2-/-	Decreased bone formation	Brommage et al. [17]
SERT-/-	Decreased bone formation	Warden et al. [35]
5-HT1A	Expressed on osteoblasts and osteoclasts; action not yet identified	Bliziotes et al. [34][a]
		Bliziotes et al. [42][a]
5-HT1B-/-	Increased osteoblast proliferation	Yadav et al. [21]
5-HT2A inhibition	Decreased osteoblast differentiation	Hirai et al. [41][a]
		Tanaka et al. [45]
5-HT2B-/-	Reduced bone formation	Collet et al. [44]
5-HT2B-/-	Impaired osteoblast proliferation, recruitment, and matrix mineralization	Chabbi-Achengli et al. [43]
5-HT2c-/-	Decreased bone formation and increased bone resorption	Oury et al. [18]
5-HT6 activation	Decreased osteoblast proliferation	Yun et al. [46]

[a]Indicates observations made in cell culture

Concluding Remarks

Serotonin has emerged as another key player in a list of signaling molecules to consider when contemplating the regulation of bone formation and remodeling (Table 1). Within the bone, the biosynthetic enzyme for serotonin (TPH1), receptors for mediating serotonin's actions, and the transporter for terminating its signals are all expressed by the cells that are integral to bone formation and resorption. Furthermore, serotonin arising from sources outside of the bone can impact bone density. Neural pathways in the brain that influence bone density are initiated by serotonergic projections from the brainstem to the hypothalamus, which ultimately promote bone accrual by releasing bone from the inhibitory influence of sympathetic inputs. The predominant source of serotonin in the body is the gut, and there are strong data indicating that gut-derived serotonin can have a negative impact on bone growth by inhibiting osteoblast formation.

The importance of serotonin in bone metabolism is underscored by the fact that, in animal studies, bone density can be dramatically influenced by deletion of the gene for SERT or by its pharmacological inhibition. This is supported by clinical reports of bone fragility in patients treated chronically with SSRIs. The complexity of studying the actions of serotonin on its multiple possible targets is further complicated by the presence of genetic variations of key factors including SERT and $5HT_{1B}$, which can affect the outcome in drug studies. Furthermore, inconsistencies with regard to animal models, strains, sex, ages, diets, sampling techniques, and approaches to bone density analysis have likely contributed to apparently conflicting

results in the literature. With this in mind, it is important to gain a more thorough understanding of the interactions between serotonin and the bone as drugs modulating elements of the serotonin signaling pathway (including receptor agonists and antagonists and SSRIs) are increasingly prescribed to treat depression and other disorders in youth and adults. Furthermore, a more complete understanding of the roles of serotonin in bone metabolism could lead to novel therapeutic strategies for alleviating bone pathologies.

References

1. Rosen CJ, Bouillon R, Compston JE, Rosen V, editors. Primer on the metabolic bone diseases and disorders of mineral metabolism. 8th ed. Ames: Wiley-Blackwell; 2013.
2. Dimitri P, Rosen C. The central nervous system and bone metabolism: an evolving story. Calcified tissue international. 2016.
3. Ducy P, Karsenty G. The two faces of serotonin in bone biology. J Cell Biol. 2010;191(1):7–13.
4. Erspamer V. Experimental research on the biological significance of enterochromaffin cells. Arch Fisiol. 1937:156–9.
5. Rapport MM, Green AA, Page IH. Serum vasoconstrictor, serotonin; isolation and characterization. J Biol Chem. 1948;176(3):1243–51.
6. Page IH, Rapport MM, Green AA. The crystallization of serotonin. J Lab Clin Med. 1948;33(12):1606.
7. Erspamer V, Asero B. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature. 1952;169(4306):800–1.
8. Twarog BM, Page IH. Serotonin content of some mammalian tissues and urine and a method for its determination. Am J Phys. 1953;175(1):157–61.
9. Hensler JG. Serotonin. In: Brady ST, editor. Basic neurochemistry principles of molecular, cellular, and medical neurobiology. 8th ed. Academic; Waltham, MA. 2012. p. 300–22.
10. Mawe GM, Hoffman JM. Serotonin signalling in the gut – functions, dysfunctions and therapeutic targets. Nat Rev Gastroenterol Hepatol. 2013;10(8):473–86.
11. Bertaccini G. Tissue 5-hydroxytryptamine and urinary 5-hydroxyindoleacetic acid after partial or total removal of the gastro-intestinal tract in the rat. J Physiol. 1960;153(2):239–49.
12. Stunes AK, Reseland JE, Hauso O, Kidd M, Tommeras K, Waldum HL, et al. Adipocytes express a functional system for serotonin synthesis, reuptake and receptor activation. Diabetes Obes Metab. 2011;13(6):551–8.
13. Paulmann N, Grohmann M, Voigt JP, Bert B, Vowinckel J, Bader M, et al. Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. PLoS Biol. 2009;7(10):e1000229.
14. Kim H, Toyofuku Y, Lynn FC, Chak E, Uchida T, Mizukami H, et al. Serotonin regulates pancreatic beta cell mass during pregnancy. Nat Med. 2010;16(7):804–8.
15. Chabbi-Achengli Y, Coudert AE, Callebert J, Geoffroy V, Cote F, Collet C, et al. Decreased osteoclastogenesis in serotonin-deficient mice. Proc Natl Acad Sci U S A. 2012;109(7):2567–72.
16. Yadav VK, Oury F, Suda N, Liu ZW, Gao XB, Confavreux C, et al. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. Cell. 2009;138(5):976–89.

17. Brommage R, Liu J, Doree D, Yu W, Powell DR, Melissa Yang Q. Adult Tph2 knockout mice without brain serotonin have moderately elevated spine trabecular bone but moderately low cortical bone thickness. BoneKEy Rep. 2015;4:718.
18. Oury F, Yadav VK, Wang Y, Zhou B, Liu XS, Guo XE, et al. CREB mediates brain serotonin regulation of bone mass through its expression in ventromedial hypothalamic neurons. Genes Dev. 2010;24(20):2330–42.
19. Motyl KJ, Rosen CJ. Understanding leptin-dependent regulation of skeletal homeostasis. Biochimie. 2012;94(10):2089–96.
20. Lam DD, Leinninger GM, Louis GW, Garfield AS, Marston OJ, Leshan RL, et al. Leptin does not directly affect CNS serotonin neurons to influence appetite. Cell Metab. 2011;13(5):584–91.
21. Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schutz G, et al. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell. 2008;135(5):825–37.
22. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell. 2001;107(4):513–23.
23. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, et al. High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med. 2002;346(20):1513–21.
24. Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass DA 2nd, et al. Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol. 2002;157(2):303–14.
25. Kode A, Mosialou I, Silva BC, Rached MT, Zhou B, Wang J, et al. FOXO1 orchestrates the bone-suppressing function of gut-derived serotonin. J Clin Invest. 2012;122(10):3490–503.
26. Yadav VK, Balaji S, Suresh PS, Liu XS, Lu X, Li Z, et al. Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. Nat Med. 2010;16(3):308–12.
27. Inose H, Zhou B, Yadav VK, Guo XE, Karsenty G, Ducy P. Efficacy of serotonin inhibition in mouse models of bone loss. J Bone Miner Res Off J Am Soc Bone Miner Res. 2011;26(9):2002–11.
28. Cui Y, Niziolek PJ, MacDonald BT, Zylstra CR, Alenina N, Robinson DR, et al. Lrp5 functions in bone to regulate bone mass. Nat Med. 2011;17(6):684–91.
29. Cui Y, Niziolek PJ, MacDonald BT, Alenina N, Matthes S, Jacobsen CM, et al. Reply to Lrp5 regulation of bone mass and gut serotonin synthesis. Nat Med. 2014;20(11):1229–30.
30. Kode A, Obri A, Paone R, Kousteni S, Ducy P, Karsenty G. Lrp5 regulation of bone mass and serotonin synthesis in the gut. Nat Med. 2014;20(11):1228–9.
31. Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. Gene. 2012;492(1):1–18.
32. Warden SJ, Robling AG, Haney EM, Turner CH, Bliziotes MM. The emerging role of serotonin (5-hydroxytryptamine) in the skeleton and its mediation of the skeletal effects of low-density lipoprotein receptor-related protein 5 (LRP5). Bone. 2010;46(1):4–12.
33. de Vernejoul MC, Collet C, Chabbi-Achengli Y. Serotonin: good or bad for bone. BoneKEy Rep. 2012;1:120.
34. Bliziotes MM, Eshleman AJ, Zhang XW, Wiren KM. Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. Bone. 2001;29(5):477–86.
35. Warden SJ, Bliziotes MM, Wiren KM, Eshleman AJ, Turner CH. Neural regulation of bone and the skeletal effects of serotonin (5-hydroxytryptamine). Mol Cell Endocrinol. 2005;242(1–2):1–9.
36. Warden SJ, Nelson IR, Fuchs RK, Bliziotes MM, Turner CH. Serotonin (5-hydroxytryptamine) transporter inhibition causes bone loss in adult mice independently of estrogen deficiency. Menopause (New York, NY). 2008;15(6):1176–83.
37. Westbroek I, van der Plas A, de Rooij KE, Klein-Nulend J, Nijweide PJ. Expression of serotonin receptors in bone. J Biol Chem. 2001;276(31):28961–8.
38. Raap DK, Van de Kar LD. Selective serotonin reuptake inhibitors and neuroendocrine function. Life Sci. 1999;65(12):1217–35.

39. Le Poul E, Boni C, Hanoun N, Laporte AM, Laaris N, Chauveau J, et al. Differential adaptation of brain 5-HT1A and 5-HT1B receptors and 5-HT transporter in rats treated chronically with fluoxetine. Neuropharmacology. 2000;39(1):110–22.

40. Bliziotes M, Gunness M, Eshleman A, Wiren K. The role of dopamine and serotonin in regulating bone mass and strength: studies on dopamine and serotonin transporter null mice. J Musculoskelet Neuronal Interact. 2002;2(3):291–5.

41. Hirai T, Tokumo K, Tsuchiya D, Nishio H. Expression of mRNA for 5-HT2 receptors and proteins related to inactivation of 5-HT in mouse osteoblasts. J Pharmacol Sci. 2009;109(2):319–23.

42. Bliziotes M, Eshleman A, Burt-Pichat B, Zhang XW, Hashimoto J, Wiren K, et al. Serotonin transporter and receptor expression in osteocytic MLO-Y4 cells. Bone. 2006;39(6):1313–21.

43. Chabbi-Achengli Y, Launay JM, Maroteaux L, de Vernejoul MC, Collet C. Serotonin 2B receptor (5-HT2B R) signals through prostacyclin and PPAR-ss/delta in osteoblasts. PLoS One. 2013;8(9):e75783.

44. Collet C, Schiltz C, Geoffroy V, Maroteaux L, Launay JM, de Vernejoul MC. The serotonin 5-HT2B receptor controls bone mass via osteoblast recruitment and proliferation. FASEB J Off Publ Fed Am Soc Exp Biol. 2008;22(2):418–27.

45. Tanaka K, Hirai T, Ishibashi Y, Izumo N, Togari A. Modulation of osteoblast differentiation and bone mass by 5-HT2A receptor signaling in mice. Eur J Pharmacol. 2015;762:150–7.

46. Yun HM, Park KR, Hong JT, Kim EC. Peripheral serotonin-mediated system suppresses bone development and regeneration via serotonin 6 G-protein-coupled receptor. Sci Rep. 2016;6:30985.

47. Cui C, Kaartinen MT. Serotonin (5-HT) inhibits factor XIII-A-mediated plasma fibronectin matrix assembly and crosslinking in osteoblast cultures via direct competition with transamidation. Bone. 2015;72:43–52.

48. Warden SJ, Fuchs RK. Do selective serotonin reuptake inhibitors (SSRIs) cause fractures? Curr Osteoporos Rep. 2016;14(5):211–8.

49. Rizzoli R, Cooper C, Reginster JY, Abrahamsen B, Adachi JD, Brandi ML, et al. Antidepressant medications and osteoporosis. Bone. 2012;51(3):606–13.

50. Mezuk B, Eaton WW, Golden SH. Depression and osteoporosis: epidemiology and potential mediating pathways. Osteoporos Int: J Established Results Cooperation Between Eur Found Osteoporos Natl Osteoporos Found USA. 2008;19(1):1–12.

51. Dubnov-Raz G, Hemilä H, Vurembrand Y, Kuint J, Maayan-Metzger A. Maternal use of selective serotonin reuptake inhibitors during pregnancy and neonatal bone density. Early Hum Dev. 2012;88(3):191–4.

52. Weintrob N, Cohen D, Klipper-Aurbach Y, Zadik Z, Dickerman Z. Decreased growth during therapy with selective serotonin reuptake inhibitors. Arch Pediatr Adolesc Med. 2002;156(7):696–701.

53. Warden SJ, Robling AG, Sanders MS, Bliziotes MM, Turner CH. Inhibition of the serotonin (5-hydroxytryptamine) transporter reduces bone accrual during growth. Endocrinology. 2005;146(2):685–93.

54. Blazevic S, Erjavec I, Brizic M, Vukicevic S, Hranilovic D. Molecular background and physiological consequences of altered peripheral serotonin homeostasis in adult rats perinatally treated with tranylcypromine. J Physiol Pharmacol: Off J Pol Physiol Soc. 2015;66(4):529–37.

55. Bab I, Yirmiya R. Depression, selective serotonin reuptake inhibitors, and osteoporosis. Curr Osteoporos Rep. 2010;8(4):185–91.

56. Yirmiya R, Goshen I, Bajayo A, Kreisel T, Feldman S, Tam J, et al. Depression induces bone loss through stimulation of the sympathetic nervous system. Proc Natl Acad Sci U S A. 2006;103(45):16876–81.