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REVIEW

Serotonin: good or bad for bone

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Besides its action as a neurotransmitter, serotonin has multiple physiological functions in several peripheral organs. Recently, Yadav *et al.* suggested that peripheral serotonin produced in the gut was a major negative regulator of osteoblast proliferation. These data were challenged by Cui *et al.* that showed no change in bone density in mature mice with a global invalidation of tryptophan hydroxylase 1, the enzyme responsible of serotonin synthesis in the periphery. In this context, we showed that osteoclasts are able to synthetize serotonin that acts locally to induce osteoclast precursors differentiation. Our data and previous results from others suggest that rather than acting as a hormone, serotonin produced in the bone could act locally on osteoclast and osteoblast realizing in the bone a complete micro-serotoninergic system.

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Introduction

Serotonin or 5-hydroxytryptamine (5-HT) and its receptors are important in the regulation of all brain functions and dysregulations of the serotonin system, and have been implicated in the pathogenesis of many psychiatric and neurological disorders. Serotonin also appears to act outside the central nervous system, and has an important role in the energy balance, food intake, gastrointestinal function, and in cardiovascular and pulmonary disorders. Recently, the bone has also emerged as a target for serotonin. This has resulted in a multiplicity of data that it has been difficult to reconcile. Before focusing on this aspect, we will briefly review some of the relevant ideas about the serotonin system.

The Serotoninergic System at the Cellular Level

Serotonin is synthesized from tryptophan in two steps: the first step is rate-limiting, and depends on the enzyme tryptophan hydroxylase (TPH). Ten years ago it was discovered that there are two isoforms of this enzyme.[1,2] TPH_2 is expressed by serotonergic neurons in the raphe nuclei that project into all brain areas, and by the myenteric neurons in the gut (serotoninergic neurons of the mesenteric plexus). *Tph*$_1$ is expressed in peripheral tissues, mainly in the enterochromaffin cells of the gut. In the brain, the pineal gland synthesizes melatonin by degrading serotonin, and also contains TPH_1. This means that it is not entirely accurate to view TPH_1 and TPH_2 as a characteristic of the brain and periphery, respectively. 5-HT does not readily cross the blood–brain barrier, and so centrally and peripherally synthesized 5-HT can function independently.

The serotonin transporter (SERT or 5-HTT) regulates 5-HT transport across the cytoplasmic membrane, but does not itself directly induce any intracellular signalling (**Figure 1**). It is encoded by the *SLC6A4* gene, and belongs to a family of Na^+/Cl^--dependent exchangers. It actively transports extracellular 5-HT into cells using transmembrane gradients and an internal negative membrane potential. SERT induces the intracellular uptake of serotonin as part of a mechanism to reduce extracellular serotonin, thus reducing binding to the transmembrane receptor of the neighbouring cells. SERT is expressed not only in the brain but also in platelets and several other peripheral cells. Polymorphism of SERT has been associated with various disorders, and is targeted by a class of antidepressant drugs (SERT inhibitors (SSRIs)) that antagonize SERT. Once inside the cell, serotonin is stored in granules via a transporter, the vesicular monoamine transporter. Inside cells, 5-HT is metabolized by a monoamine oxidase to form an inactive metabolite (**Figure 1**).

Serotonin acts on different cells via 15 different transmembrane receptors (5-HTRs) that have been divided into seven classes (**Figure 1**). The diversity of action of serotonin results from the multiplicity of its receptors. Most receptors are coupled to seven transmembrane domain G protein. Cloning of these receptors has led to a better understanding of the multiple actions of serotonin, and the development of several specific agonists and antagonists used in several pathologies.[3]

Interestingly, serotonin can also signal via a receptor-independent mechanism. The intracellular concentration of serotonin depends on two mechanisms. On the one hand, some cells have an active TPH and can synthesize serotonin and, on the other, the SERT induces the uptake of

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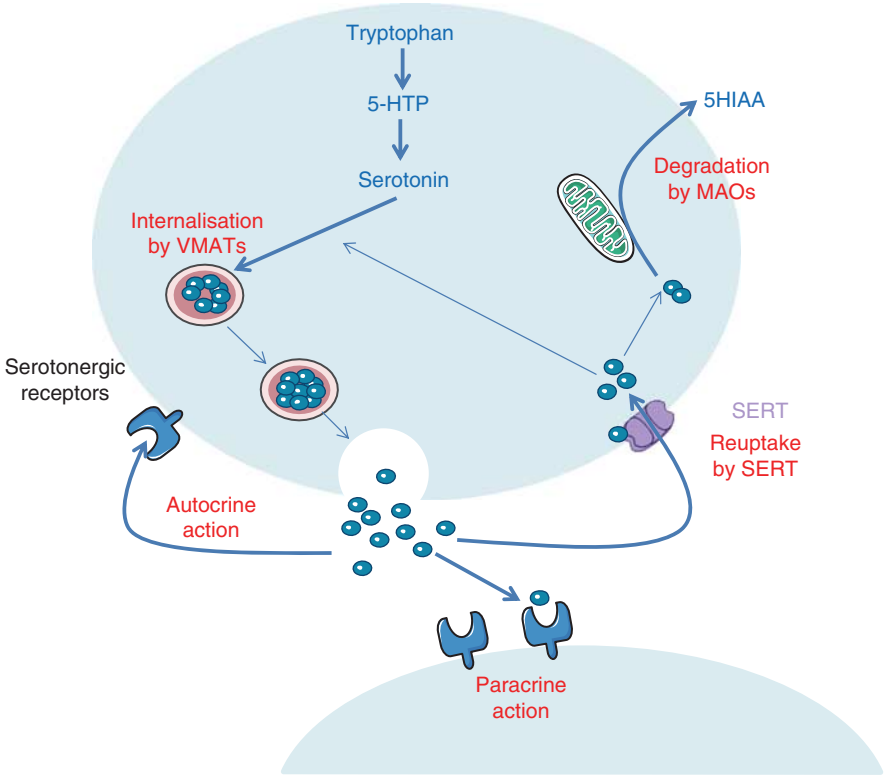

Figure 1 The serotonin system in a peripheral tissue.

extracellular serotonin. Identifying the presence of intracellular serotonin has raised the question of its function. Cells that possess the enzyme transglutaminase can covalently attach 5-HT to the glutamine residue of small GTPase to form a glutamyl-amide bond (serotonylation), resulting in the activation of these G proteins. This was demonstrated for the first time in platelets that have a high (50 mM) intracellular concentration of serotonin. Serotonylation of small GTPase induces exocytosis of alpha granules and platelet aggregation.[4]

Serotonin at the Periphery in its Context

Most serotonin is found outside of the central nervous system, mainly in the platelets, and all 15 receptors are expressed peripherally. Peripheral serotonin is mainly synthesized in the gastrointestinal tract by TPH_1: 95% of total body serotonin is released into the gut by enterochromaffin cells, where the level of expression of *Tph*$_1$ is higher.[1] Serotonin in the gut is also produced by myenteric serotonergic neurons by TPH_2, and regulates intestinal motility and mucosal secretion by a paracrine mechanism. The release of gut serotonin is mainly triggered by mechanical and chemical stimulation owing to food.[5]

Serotonin is involved in cardiovascular physiology and can induce vasoconstriction or vasodilatation, depending on the receptor expressed in the vessel wall. In cardiovascular pathology, serotonin has been implicated in the development of pulmonary hypertension and cardiac valve thickening. These effects have recently been highlighted, because of their occurrence in patients taking anorexic drugs that activate serotonin 2B receptors ($5\text{-}HT_{2B}R$).[3,6]

Platelet activation and serotonin release are involved in thrombus formation, and also in inflammation and tissue damage. Serotonin released from platelets after an injury can induce liver regeneration.[7] Although multiple peripheral actions of serotonin have been reported,[3] it is interesting to observe that if they are not challenged, *Tph*$_1$-invalidated mice have a limited phenotype: reduced cardiac contractibility,[8] slower gut motility,[9] decreased platelet aggregation[4] and anaemia.[10]

It is important to observe that there are other peripheral sites of serotonin synthesis in addition to the gut. Serotonin has been shown to be synthesized in the mammary gland, and has been implicated in the feedback autocrine paracrine mechanism of lactation.[11] It is also synthesized in the keratinocyte, where it is degraded to form melatonin.[12] In the vascular system it is synthesized by endothelial cell, and can induce proliferation of the smooth muscular cells of the arteries.[13] Finally, it is produced by the beta cell of the pancreas, and induces insulin exocytosis by serotonylation of GTPase rab3a and rab27a.[14] In these various cells, serotonin usually acts both by serotonylation of the small G protein that induces exocytosis or contraction, and by receptor-mediated intracellular signalling. In all these examples, the concept of a micro-serotoninergic system is more convincing than considering 5-HT as a pleiotropic hormone.

The Earlier *In Vitro* Studies of Serotonin and the Bone

Several *in vitro* studies have reported the existence of a serotonin receptor and/or of a functional SERT in primary bone cells or in bone cell lines. In the osteoblast and osteoblast cell lines, it is mainly $5\text{-}HT_{1A}R$, $5\text{-}HT_{2A}R$ and $5\text{-}HT_{2B}R$ protein expression and/or binding sites that have been observed.[15,16] A proliferative

action on serotonin on chicken osteoblast, mimicked by a 5-HT_{2B}R agonist, has been described. This study was performed with a physiological concentration of serotonin (1–1000 nM) in medium that had not been serotonin depleted. Osteocytic cell line MLO-Y4 has 1A and 2A receptors.[17] Several studies have also revealed the existence of a functional transporter in both the osteoblast and osteoclast cell lines.[17,18] However, these pioneering studies could not provide any clue about the physiological role of serotonin in bone remodelling *in vivo*. Furthermore, the origin of the serotonin in the bone microenvironment was not clear. There are no serotonergic nerves in the bone, nor in any other peripheral tissue except the gut. At this point, Yadav *et al.*[19] suggested that 'free' circulating serotonin produced by the gut was responsible for the action of serotonin on bone cells.

SSRIs and Osteoporosis

The first *in vivo* study to investigate the serotoninergic system and the bone that had a significant impact on the understanding of human pathology was that of Warden *et al.*[20] They reported a massive deficit in bone acquisition in SERT-invalidated mice, and also in mice treated with fluoxetine, a SSRI used to treat depression. This bone phenotype was related to a decrease in bone formation.[20] It should be observed that clinically relevant doses of fluoxetine induce only moderate bone architecture changes in rats [21] Although SERT is functional in osteoblasts,[15] the mechanism responsible for the decreased osteoblast function was not clear, and no *ex vivo* experiments on osteoblasts have been performed. No known extraskeletal action of SERT could explain the decrease in bone accrual. Alongside this experimental work, several studies have tried to evaluate the impact on osteoporosis of SSRIs that are widely used to treat depression. In contrast to the massive phenotype observed in growing mice, retrospective studies evaluating bone density in adult patients treated with SSRIs have provided mixed conclusions, with some studies showing no change in bone density and others a decrease.[22–24] With regard to fractures, most of the data showed a significant increase in odds ratio for fractures in patients treated with SSRIs.[22,23] Most of the studies were adjusted for the presence of depression, which has been shown to increase the risk of fractures.[22] For most investigators, the role of SSRI in osteoporosis is still an open question.[25] It is noteworthy that, as far as we are aware, there is no study evaluating bone remodelling markers in patients treated with an SSRI.

Serotonin and the Bone: the Controversy

Respective roles of serotonin and LRP5. In a paper that has led to considerable rethinking,[26,27] Yadav *et al.*[19] demonstrated that circulating serotonin reduced osteoblast proliferation, and that this depended on the 1_B receptor on osteoblasts. Most of the interest created by this innovative study was driven by the hypothesis that the role of LRP5 in the bone is not owing to the expression of this gene in cells of the osteoblast lineage, but is dependent on the synthesis of serotonin by the gut, which is regulated by LRP5. Several new observations about the physiology of serotonin were made in this paper. It is interesting to note that these authors pointed out that the TPH_1 enzyme is regulated by the *Lrp5* gene, because hitherto no known transcriptional regulation of *Tph*$_1$ had been reported. Surprisingly, considering the usual role of serotonin in cell replication,[7,16,28–31] serotonin decreased osteoblast proliferation. However, the concentrations used were 500 times higher than physiological concentrations. This paper was mainly concerned with specific *Tph*$_1$ invalidation in the gut, and mice in which the different serotonin receptors in osteoblasts had been invalidated, but which had not undergone global *Tph*$_1$ invalidation. It was also surprising that these authors observed an increase in bone density in mice in which the 1_B receptor had been specifically inactivated in the osteoblasts, whereas binding sites for this receptor could not be detected in osteoblast primary cultures.[32] Yadav *et al.*[33] also showed that a specific TPH_1 inhibitor, LP533401, could increase bone density through an anabolic action that was as potent as that of teriparatide in ovariectomized mice and rats. In 2011, Cui *et al.*[34] published a demonstration of the direct action of LRP5 in cells of the osteoblast lineage, namely osteocytes. They showed that an *Lrp5* knock-in mutation inducing high bone mass syndrome in osteocytes also induced increased bone density. They also showed that mice with a global *Tph*$_1$ knockout showed no change in bone density at 4 months of age, but the authors did not study bone remodelling in these mice. Finally, Cui *et al* observed that a small-molecule inhibitor of TPH_1, LP923941, which is an enantiomer of LP533401 used by Yadav *et al*, lowered circulating 5-HT, but did not change the bone density of the mice. Some of the discrepancies between the data reported by these two groups could be owing to differences in the promoters used to drive the expression of the transgene or to localize cre recombinase in these tissue-specific knock-in.[25] The different mice models used in these two papers are recapitulated in the **Table 1**. It appears that different mice models were used and this might explain the discrepancies between both studies. Notably, osteoblast specificity was obtained using different promoters. The only divergent data concern the results obtained using the villin promoter but a different cre mice lines was used in each study.

Finally in another controversial point, Yadav *et al* reported elevated levels of serum serotonin in mice with a global *Lrp5,* and in patients with osteoporosis pseudoglioma that is related to homozygous inactivating mutation of *Lrp5*. Cui *et al* observed no difference in serum serotonin levels in mice with the different mutations of the *Lrp5* gene in their osteocytes.

Blood levels of serotonin and the bone. Most of the serotonin present in blood is stored in the platelets, and is not biologically active in the absence of platelet activation. Platelets do not synthesize serotonin, which is internalized by the SERT and then stored in dense granules and released during platelet activation or lysis. Measurement of whole blood or serum levels of serotonin mainly reflects the number of platelets as the concentration of serotonin in individual platelets is fairly constant. Therefore the physiological relevance of studies in which serum serotonin was measured and correlated with bone mineral density[35] or determined in patients with *Lrp5* mutations[36] is rather unclear. The amount of free serotonin in platelet-poor plasma is extremely low, between 2 and 10 nM, whereas serotonin levels in whole blood are 50 times higher. The only human disorder in which serotonin, like other peptides, is markedly elevated in both platelets and plasma is carcinoid syndrome, where values are 10- to 100-fold higher than the normal range owing to massive secretions from enterochromaffin cells. However, osteoporosis and change in bone remodelling markers have not been observed in these patients who have a long survival.[37,38]

Table 1 Mice model in Yadav *et al.* and Cui *et al.* studies

	Yadav et al.[19]	*Cui et al.*[34]
Lrp 5 knockout mice (*lrp5 −/−*)	*Lrp5 −/− Global KO:* ↓ bone formation *in vivo* ↓ osteoblast proliferation *ex vivo* = osteoblast proliferation *Lrp5 −/− in osteoblast (Col-I Cre):* = bone mass *Lrp5 −/− in gut (villin Cre):* ↓ bone mass, with ↓ osteoblast number and = osteoclast	*Lrp5 −/− in osteocytes (Dpm-1 Cre):* ↓ bone mass *Lrp5 −/− in gut (villin Cre):* = bone mass
Lrp5 knock-in mice (*lrp5*KI)		*Lrp5 KI in osteocytes (Dpm-1 Cre):* ↑ bone mass *Lrp5 KI in gut (villin Cre):* = bone mass
Tph1 knockout mice (*tph1 −/−*)	*Tph1 −/− KI in gut (villin Cre)* ↑ bone mass with ↑ osteoblast number and = osteoclast number *Tph1 −/− in osteoblast (Col-I Cre):* = bone mass	*Tph1 −/− Global KO from 12 weeks:* = bone mass

By contrast, rats daily injected during 6 months with serotonin, inducing high circulating serotonin level, had a high bone mass with increased bone formation.[39]

As expected, SERT is an important determinant of blood serotonin levels as it is required for platelet storage. SERT-invalidated mice have low platelet levels of serotonin, but also in plasma, as a result of increased degradation. No long-term change in circulating level of serotonin is reported in patients treated with SSRIs.

Serotonin receptors in osteoblasts. In earlier works, it has already been shown that osteoblasts have several serotonin receptors, and here we investigated the bone phenotype of mice with a global invalidation of the serotonin 2B receptor (*htr2b*$^{-/-}$). In all, 30% of newborn *htr2b*$^{-/-}$ mice die at birth with cardiac malformation, but the survivors have normal life expectancy and display only minimal cardiac dysfunction.[40] We have previously shown that mice displayed significantly reduced bone formation from 4 months of age.[32] This osteopenia was intensified in 18-month-old mice that displayed a phenotype reminiscent of aging-related osteoporosis. This could not be detected in 3-month-old mice with an osteoblast-specific deletion of this serotonin 2B receptor studied by Yadav *et al.*[19] In the absence of serotonin, osteoblasts of 5-HT_{2B}R −/− mice have reduced bone proliferation. Our pharmacological data suggest that this 2B receptor is capable of both constitutive and paracrine activities in accordance with the data obtained by Locker *et al.*[41] on a mesoblastic cell line. The only other serotonin receptors known to have binding sites on osteoblasts were 5-HT_{2A}R and 5-HT_{1A}R. Several points need to be considered: first, although osteoblasts do express *Tph*$_1$ transcriptionally, we could not detect any significant serotonin synthesis.[42] Second, we observed that 10–100 nM serotonin has a proliferative action on primary murine osteoblasts, which is consistent with previous data for chicken osteoblasts,[16] whereas an antiproliferative effect could be observed from 50 μM (unpublished data).

Serotonin and osteoclasts. In order to investigate in greater depth the role of the serotoninergic system in bone remodelling, we conducted a detailed *in vivo* study in *Tph*$_1^{-/-}$ mice. We studied male mice while they were growing (six weeks of age) and at maturity (16 weeks).[42] In the growing *Tph*$_1^{-/-}$ mice, we observed an increase in bone mass that was resolved at maturity, in accordance with the data of Cui *et al.*[34] In both juvenile and mature mice there was evidence of decreased bone resorption, as evaluated by both histomorphometry and *D*-pyridinoline, a biochemical marker of bone resorption. Bone formation was unchanged in growing mice and reduced in mature mice, which explains the elevated bone density seen in the growing mice that had returned to normal at maturity. We also showed that *in vivo* the decrease in bone resorption could be overridden by treatment with 5-HTP, which offsets the absence of TPH_1. Furthermore, bone-marrow transplantation at birth of *Tph*$_1^{-/-}$ mice with wild-type cells retarded the deficit in bone resorption and proved that an intrinsic osteoclast defect was responsible for the osteoclast phenotype. *Ex vivo* studies showed that osteoclast differentiation from spleen cells (in the absence of any osteoblastic cell of the osteoblast lineage) was reduced in *Tph*$_1^{-/-}$ animals. Finally, we could show that osteoclasts were able to synthesize serotonin, and that this synthesis was increased by RANKL. These data could not have been observed by Yadav *et al*, as they reported only specific invalidation of *Tph*$_1$ in the gut.

This result shows for the first time that there is a source of serotonin in the bone microenvironment. This synthesis of serotonin by osteoclasts might increase in a pathological situation where there is an increase in RANKL. Although our data show that serotonin increases osteoclastogenesis by a paracrine/autocrine mechanism, the physiological role of serotonin in osteoblastogenesis is less clear. It is possible that osteoclast synthesis of serotonin is sufficient to induce an increase in osteoblast proliferation, and that serotonin is one of the 'coupling factors' that are synthesized by osteoclasts and stimulate osteoblast formation.[43] However, this hypothesis cannot explain why normal bone formation was maintained in growing mice despite the decreased bone resorption in *Tph*$_1^{-/-}$ mice.

Avenues of Research Concerning Serotonin and the Bone

The hypothetical model in which serotonin acts as a pleiotropic hormone on bone cells appears less plausible in the bone, as in other peripheral organs. From the different studies of the action of serotonin on the bone, bone can be viewed as a micro-serotoninergic system with a paracrine/autocrine system originating from osteoclasts acting on osteoclasts themselves and also on osteoblasts (**Figure 2**). There is currently no known example of the regulation of this local system by cytokines or systemic hormones. Low amounts of serotonin present in the bone microenvironment produced by osteoclasts would trigger activated proliferation of both osteoclast and osteoblast precursors. The reality is certainly more complex, and many questions, mainly concerning the role of SERT, are still awaiting answers:

- How does SERT influence osteoblasts? Osteoblasts have a functional SERT, but how the SERT signals to osteoblasts so far remains to be elucidated.

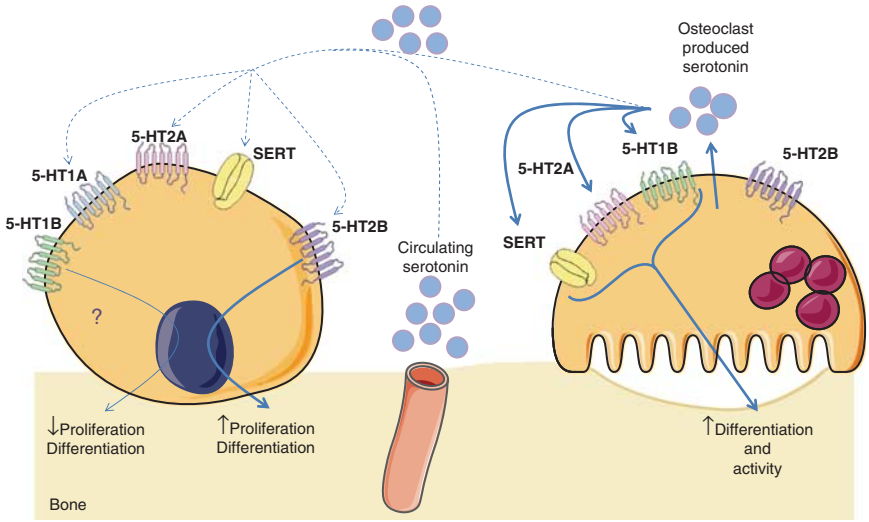

Figure 2 Hypothesis on the role of serotonin on bone cells.

- What bone remodelling changes, if any, occur in patients taking SSRIs?
- What are the roles of the small amount of serotonin synthesised by the osteoclasts versus the circulating serotonin in bone?
- Does the serotonin released from platelets have any role in calcifications in arteries and valves?

Like other neuropeptides and bioamines, serotonin is one of the local factors in physiological bone remodelling. The reason why it is particularly important to investigate serotonin is the huge number of patients who are currently treated with agonists or antagonists of serotonin receptors or with drugs impacting on the SERT. It is therefore important that clinical and experimental studies using different models should be carried out to provide answers to these questions.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA *et al.* Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc Natl Acad Sci USA* 2003;**100**:13525–13530.
2. Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H *et al.* Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 2003;**299**:76.
3. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. *Annu Rev Med* 2009;**60**: 355–366.
4. Walther DJ, Peter JU, Winter S, Holtje M, Paulmann N, Grohmann M *et al.* Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell* 2003;**115**:851–862.
5. Spiller R. Serotonin and GI clinical disorders. *Neuropharmacology* 2008;**55**:1072–1080.
6. Nebigil CG, Choi DS, Dierich A, Hickel P, Le Meur M, Messaddeq N *et al.* Serotonin 2B receptor is required for heart development. *Proc Natl Acad Sci USA* 2000;**97**:9508–9513.
7. Gustafsson BI, Thommesen L, Stunes AK, Tommeras K, Westbroek I, Waldum HL *et al.* Serotonin and fluoxetine modulate bone cell function *in vitro*. *J Cell Biochem* 2006;**98**:139–151.
8. Fligny C, Fromes Y, Bonnin P, Darmon M, Bayard E, Launay JM *et al.* Maternal serotonin influences cardiac function in adult offspring. *FASEB J* 2008;**22**:2340–2349.
9. Ghia JE, Li N, Wang H, Collins M, Deng Y, El-Sharkawy RT *et al.* Serotonin has a key role in pathogenesis of experimental colitis. *Gastroenterology* 2009;**137**:1649–1660.
10. Amireault P, Hatia S, Bayard E, Bernex F, Collet C, Callebert J *et al.* Ineffective erythropoiesis with reduced red blood cell survival in serotonin-deficient mice. *Proc Natl Acad Sci USA* 2011; **108**:13141–13146.
11. Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M *et al.* Serotonin regulates mammary gland development via an autocrine-paracrine loop. *Dev Cell* 2004;**6**: 193–203.
12. Slominski A, Semak I, Pisarchik A, Sweatman T, Wortsman J, Szczesniewski A *et al.* Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. *FEBS Lett* 2002;**511**: 102–106.
13. Eddahibi S, Guignabert C, Barlier-Mur AM, Dewachter L, Fadel E, Dartevelle P *et al.* Cross talk between endothelial and smooth muscle cells in pulmonary hypertension: critical role for serotonin-induced smooth muscle hyperplasia. *Circulation* 2006;**113**:1857–1864.
14. 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**:e1000229.
15. 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**:477–486.
16. 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**:28961–28968.
17. 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**: 1313–1321.
18. Battaglino R, Fu J, Spate U, Ersoy U, Joe M, Sedaghat L *et al.* Serotonin regulates osteoclast differentiation through its transporter. *J Bone Miner Res* 2004;**19**:1420–1431.
19. Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schütz G *et al.* Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell* 2008;**135**:825–837.
20. 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**:685–693.
21. Westbroek I, Waarsing JH, van Leeuwen JP, Waldum H, Reseland JE, Weinans H *et al.* Long-term fluoxetine administration does not result in major changes in bone architecture and strength in growing rats. *J Cell Biochem* 2007;**101**:360–368.
22. Spangler L, Scholes D, Brunner RL, Robbins J, Reed SD, Newton KM *et al.* Depressive symptoms, bone loss, and fractures in postmenopausal women. *J Gen Intern Med* 2008;**23**: 567–574.
23. Richards JB, Papaioannou A, Adachi JD, Joseph L, Whitson HE, Prior JC *et al.* Effect of selective serotonin reuptake inhibitors on the risk of fracture. *Arch Intern Med* 2007;**167**: 188–194.
24. Haney EM, Chan BK, Diem SJ, Ensrud KE, Cauley JA, Barrett-Connor E *et al.* Association of low bone mineral density with selective serotonin reuptake inhibitor use by older men. *Arch Intern Med* 2007;**167**:1246–1251.
25. Goltzman D. LRP5, serotonin, and bone: complexity, contradictions, and conundrums. *J Bone Miner Res* 2011;**26**:1997–2001.
26. Rosen CJ. Breaking into bone biology: serotonin's secrets. *Nat Med* 2009;**15**:145–146.
27. Rosen CJ. Serotonin rising–the bone, brain, bowel connection. *N Engl J Med* 2009;**360**: 957–959.
28. Banes AK, Shaw SM, Tawfik A, Patel BP, Ogbi S, Fulton D *et al.* Activation of the JAK/STAT pathway in vascular smooth muscle by serotonin. *Am J Physiol Cell Physiol* 2005;**288**: C805–C812.
29. Nemecek GM, Coughlin SR, Handley DA, Moskowitz MA. Stimulation of aortic smooth muscle cell mitogenesis by serotonin. *Proc Natl Acad Sci USA* 1986;**83**:674–678.
30. Pakala R, Willerson JT, Benedict CR. Mitogenic effect of serotonin on vascular endothelial cells. *Circulation* 1994;**90**:1919–1926.

31. Seuwen K, Magnaldo I, Pouyssegur J. Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT1B receptors coupled to a Gi-protein. *Nature* 1988;**335**:254–256.
32. 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* 2008;**22**:418–427.
33. 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**:308–312.
34. 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**:684–691.
35. Modder UI, Achenbach SJ, Amin S, Riggs BL, Melton 3rd LJ, Khosla S *et al.* Relation of serum serotonin levels to bone density and structural parameters in women. *J Bone Miner Res* 2010;**25**:415–422.
36. Frost M, Andersen T, Gossiel F, Hansen S, Bollerslev J, van Hul W *et al.* Levels of serotonin, sclerostin, bone turnover markers as well as bone density and microarchitecture in patients with high-bone-mass phenotype due to a mutation in Lrp5. *J Bone Miner Res* 2011;**26**:1721–1728.
37. Kulke MH, Mayer RJ. Carcinoid tumors. *N Engl J Med* 1999;**340**:858–868.
38. van Dijk SC, de Herder WW, Kwekkeboom DJ, Zillikens MC, Feelders RA, van Schaik RH *et al.* 5-HIAA excretion is not associated with bone metabolism in carcinoid syndrome patients. *Bone* 2012;**50**:1260–1265.
39. Gustafsson BI, Westbroek I, Waarsing JH, Waldum H, Solligard E, Brunsvik A *et al.* Long-term serotonin administration leads to higher bone mineral density, affects bone architecture, and leads to higher femoral bone stiffness in rats. *J Cell Biochem* 2006;**97**:1283–1291.
40. Nebigil CG, Hickel P, Messaddeq N, Vonesch JL, Douchet MP, Monassier L *et al.* Ablation of serotonin 5-HT(2B) receptors in mice leads to abnormal cardiac structure and function. *Circulation* 2001;**103**:2973–2979.
41. Locker M, Bitard J, Collet C, Poliard A, Mutel V, Launay JM *et al.* Stepwise control of osteogenic differentiation by 5-HT(2B) receptor signaling: nitric oxide production and phospholipase A2 activation. *Cell Signal* 2006;**18**:628–639.
42. 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 USA* 2012;**109**:2567–2572.
43. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc Natl Acad Sci USA* 2008;**105**:20764–20769.