

Serotonin Rising

TO THE EDITOR: Rosen's Perspective article (March 5 issue)¹ highlights recent findings that gut-derived serotonin inhibits bone formation by stimulating serotonin receptors on the preosteoblast.² A critical question is whether serotonin is delivered to bone in some blood element or as free plasma serotonin. The serum serotonin measurements used by Yadav and colleagues² reflect an undefined proportion of the platelet pool and say nothing about the minuscule and often mismeasured free plasma concentrations.³

If the platelet is the delivery vehicle, it is paradoxical that increased platelet serotonin levels in Lrp5-knockout animals and patients with osteoporosis pseudoglioma lead to bone loss,² whereas treatment with selective serotonin-reuptake inhibitors (SSRIs), which lowers platelet serotonin levels by 80 to 95%, also reduces bone mass.⁴ The apparent requirement for maternally derived serotonin in mammalian embryogenesis⁵ poses a similar puzzle: How can gestational SSRI treatment markedly reduce maternal platelet serotonin levels without disrupting embryonic development? Perhaps local tissue uptake and release are crucial in regulating exposures. Finally, given the apparent inhibitory role of serotonin (however delivered) in bone formation,^{1,2} it is puzzling that the carcinoid syndrome has not been commonly associated with osteoporosis.

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TO THE EDITOR: Do carcinoids shed light on serotonin-induced osteoporosis? Yadav et al.¹ discovered that duodenal-derived circulating serotonin (whether in platelets or free is unspecified) inhibits bone formation. In his Perspective article, Rosen interprets this finding as an explanation for SSRI-induced osteoporosis. SSRIs significantly reduce circulating serotonin by inhibiting platelet uptake of serotonin as a consequence of blocking the serotonin transporter. Normally, circulating serotonin is almost entirely confined to platelets. We do not know the levels of free serotonin in patients taking SSRIs, but they would be expected to be elevated.

We detected highly elevated levels of serotonin in platelets and free plasma in patients with serotonin-producing metastatic carcinoid tumors, with a median level of free serotonin of 82.1 nmol per liter (as compared with 4.0 nmol per liter among healthy control subjects) and a median level of platelet serotonin of 18.0 nmol per 1×10⁹ platelets, as compared with 3.4 nmol per 1×10⁹ platelets among control subjects.^{2,3} However, there are no obvious leads pointing to osteoporosis in such patients. Even in cases of bone metastases, we observed no changes in patients' bone-metabolism markers.⁴

This discrepancy may well be due to the fact that apart from circulating serotonin, metabolic clearance plays a role. Since SSRIs also reduce serotonin clearance in peripheral transporter-expressing target organs, such as bone, serotonin-receptor activation is increased. In contrast, in patients with carcinoid tumors, transporter function is intact, and metabolic clearance can be highly up-regulated.⁴

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TO THE EDITOR: The article by Rosen contains a minor error. Tam et al. did not report the presence of cannabinoid receptor type 1 (CB1) on osteoblasts, nor was this the cause of increased bone formation after traumatic brain injury.¹ Rather, they describe CB1 receptors on presynaptic terminals of the sympathetic neurons innervating osteoblasts. The proposed mechanism for increased bone formation was inhibition of norepinephrine release from sympathetic nerve terminals by the endogenous endocannabinoid 2-arachidonoylglycerol.

The β_2 -adrenergic receptor on the osteoblasts is reported to be the target of this norepinephrine.^{1,2} It is generally understood that norepinephrine has poor affinity for the β_2 -adrenergic receptor.³ This suggests two possibilities: either the concentration of norepinephrine in the synapse is sufficiently high to activate β_2 -adrenergic receptors to cause a response in the osteoblasts or epinephrine that is taken up and recycled by sympathetic nerve terminals⁴ activates the β_2 -adrenergic receptors, leading to increased diversion of osteoblasts to osteoclasts.

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THE AUTHOR REPLIES: Speth is correct in pointing out an error in my Perspective article in regard to the relationship between CB1 receptors and osteoblast function. In the head-injury model reported by Tam et al., the CB1 receptors were on presynaptic terminals of sympathetic fibers innervating osteoblasts.¹ The proposed mechanism for the increase in bone mass in this model was the activation of cannabinoid receptor type 1 by endogenous cannabinoids, leading to the inhibition of norepinephrine release. Nevertheless, this study illustrates our growing understanding of the relationship between neuronal signaling and target cells in the bone marrow that define the rate of bone remodeling. In fact, Olmsted-Davis et al. recently reported that in an animal model of heterotopic ossification induced by bone morphogenetic protein 2, neurons and their progenitor cells appear very early and well before the vascular and osteogenic phases of new bone formation are established.² With respect to the mechanism of bone loss from sympathetic activity, activated adrenergic receptors on osteoblasts suppress critical transcription factors necessary for bone formation but also enhance osteoclastogenesis, principally by up-regulating the osteoclast differentiation factor RANKL.³ This is not a diversion of osteoblasts to osteoclasts, as noted by Speth, but rather a dynamic process of coupling that involves two cell types originating from distinct progenitor cells.

Anderson and colleagues raise an important question: How does the delivery of circulating serotonin affect bone? Clearly, there are dynamic changes in both platelet uptake and renal clearance, as noted by de Jong et al. in their letter, and these functions are significantly altered in patients receiving SSRIs. In my article, I point out that the article by Yadav et al. did not elucidate the mechanism of bone loss with these agents, particularly since this drug class has a profound effect on the reuptake of serotonin by the central nervous system.⁴ Hence, it is conceivable that there is a balance in bone turnover between the central blockade of serotonin reuptake and changes that may be associated with circulating serotonin and its release from platelets. Furthermore, as de Jong et al. state, high serotonin levels in the carcinoid syndrome did not correlate with markers of bone turnover. These data reinforce the need for com-

prehensive longitudinal studies of circulating serotonin measured in a standardized manner in patients with osteoporosis and other disorders.

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More on Iodine Content of Prenatal Vitamins

TO THE EDITOR: I have several concerns about the letter to the Editor by Leung et al. (Feb. 26 issue).¹ Scientific studies must provide methodologic details or else the veracity of the results presented cannot be evaluated. Leung et al. provide no details regarding sample preparation and the method used for detecting and quantitating iodine content. A titrimetric method for measuring iodine, which dates back to 1932,² or a modern method involving inductively coupled plasma-mass spectroscopy³ may have been used. Both methods are subject to interferences. In addition, the study draws conclusions about the iodine content in marketed products; these conclusions are not supported by a sampling plan and thus may not be justified.⁴ Tools for assessing the accuracy and precision of chemical measurements do exist; for instance, Standard Reference Material 3280 can be used to assign a certified value to the iodine content of multivitamins and multielement tablets.⁵ Although we support the need for ensuring nutritional adequacy, the lack of rigor in sampling and reporting casts doubt on the accuracy of the results of the study by Leung and colleagues.

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THE AUTHORS REPLY: With regard to the comments of Betz and Wise: our description of laboratory methods in our letter was limited because of space constraints. We measured iodine in prenatal multivitamin tablets, using a modification of the Sandell-Kolthoff spectrophotometric method originally described by Pino, from our laboratory, in 1965.¹ Our laboratory also measures iodine concentrations by means of mass spectrometry, and the results of the two methods are generally similar. Our laboratory has been certified annually by the Ensuring the Quality of Urinary Iodine Procedures (EQUIP) program of the Centers for Disease Control and Prevention, and we have measured iodine in urine and other substances for multiple studies over the past four decades.²⁻⁵

Our intent was to provide a broad overview of the supplements available to pregnant and lactating women, who may be susceptible to iodine deficiency. Among the observations we noted in our letter to the Editor was that 49% of types of prenatal multivitamins marketed in the United States contain no iodine; this finding is not dependent on laboratory or sampling methods and has important implications for public health.

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