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REVIEW PAPER

Effects of Sympathetic Activity on Human Skeletal Homeostasis: Clinical Evidence from Pheochromocytoma

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Abstract

Bone is a highly dynamic tissue that is continuously being renewed in a lifelong remodeling process that is guided by mechanical and biochemical signals. Imbalances between the underlying processes result in metabolic bone diseases, such as osteoporosis and osteopetrosis; therefore, all phases of bone remodeling are tightly controlled by systemic or local factors to maintain bone homeostasis. The sympathetic nervous system (SNS), one of the two main divisions of the autonomic nervous system, operates through a series of interconnected neurons and finalizes the actions by binding catecholamines, released from postganglionic neurons and from adrenal medulla to adrenergic receptors on peripheral target tissues. Because bone is a metabolically active organ with a significant neural innervation, it is reasonable to assume that neuronal control may constitute a key mechanism in bone remodeling. Pheochromocytoma is a neuroendocrine tumor arising from the chromaffin cells of the adrenal medulla and is characterized by an excessive production of catecholamines. Considering that catecholamines are the main neurotransmitters of SNS, pheochromocytoma might be an ideal human model to determine the role of sympathetic outflow on the pathogenesis of a variety of diseases. This review highlights the importance of SNS activity in human skeletal homeostasis based on insights gained from pheochromocytoma, combined with evidence from a variety of experimental studies.

Keywords Sympathetic nervous system · Bone remodeling · Adrenergic receptors · Bone resorption · Pheochromocytoma

Introduction

Bone is a highly dynamic tissue that undergoes continuous renewal via remodeling processes that persist throughout life and are guided by mechanical and biochemical signals. This constant remodeling occurs by means of three consecutive series of events: (a) initiation of bone resorption by osteoclasts, (b) transition from catabolism to anabolism, and (c) bone formation by osteoblasts completed by termination [1, 2]. Because imbalances between these processes may result in metabolic bone diseases, such as osteoporosis and osteopetrosis [3], all phases of bone remodeling are tightly controlled by systemic or local factors to maintain bone homeostasis.

The autonomic nervous system (ANS) mediates many of the body's involuntary actions, and the sympathetic nervous system (SNS) is one of the two main branches of the ANS, the other one being the parasympathetic nervous system. Although the SNS is well known to induce "fight-or-flight" responses to perceived threats, it innervates almost all organs and thereby enables basic homeostatic regulation in multiple ways [4]. The SNS operates through a series of interconnected neurons and finalizes the actions by binding catecholamines, released from postganglionic neurons and from adrenal medulla to adrenergic receptors (ARs) on peripheral target tissues [5]. Because bone is a metabolically active organ with a significant neuronal innervation [6, 7], it is reasonable to assume that neuronal control may be one of key mechanisms that regulate bone remodeling. This review discusses the importance of sympathetic neuronal activity in human skeletal homeostasis, based on insights gained from a clinical disease model that involves the SNS activity, as well as from experimental data from a variety of studies.

Experimental Evidence Supporting the Role of the SNS in Bone Metabolism

Although bone is a strongly innervated organ, the overall pattern of nerve distribution in bone remodeling units suggests

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that bone cells are rarely in direct contact with nerve terminals [8], which indicates the importance of neurotransmitters as mediators between SNS outflow and bone metabolism [9]. Among the five subtypes of ARs (β1, β2, β3, α1, and α2) that are activated by catecholamines, the β2AR, which shows a particularly strong expression in osteoblasts [10, 11], is regarded as the principal mediator between sympathetic neuronal activity and rodent skeletal functioning.

Pioneering research on the role of the SNS as a peripheral effector of brain leptin signaling that regulates bone physiology was performed by the Karsenty laboratory [9, 10, 12, 13]. Leptin is an adipocyte-derived hormone that inhibits appetite and increases energy expenditure primarily via the hypothalamic arcuate nucleus [14]. Mice lacking leptin or its receptor (*ob*/*ob* and *db*/*db* mice, respectively) exhibit increased bone mass by stimulated bone formation despite concurrent hypogonadism [12]. Intracerebroventricular (ICV) infusion of leptin into the third ventricle of leptin-deficient mice (*ob*/*ob* mice) fully reversed their high bone mass phenotype by inducing bone loss [12]. Moreover, mice lacking only neuronally expressed leptin receptors showed a markedly high bone mass, similar to global leptin receptor knockout mice (*db*/*db* mice); in contrast, bone-related parameters were not altered in osteoblast-specific leptin receptor-deficient mice [13]. Together, these results suggest that bone metabolism is centrally controlled by leptin. Further murine studies demonstrated that sympathetic stimulation of β2AR in bone may be a critical link between central leptin signaling and skeletal functioning. Specifically, ICV infusion of leptin failed to reduce bone mass in mice that were lacking β2AR and dopamine β-hydroxylase, an enzyme necessary for the synthesis of catecholamines [10, 15]. Moreover, the β-adrenergic agonist, isoproterenol, significantly decreased bone mass in *ob*/*ob* mice [10].

Additionally, animal studies using β-adrenergic modulators and genetic manipulation have provided compelling evidence for the role of sympathetic neuronal activity in skeletal homeostasis. Treatment with β-blockers significantly rescued bone loss in murine osteoporosis models induced by various means, including ovariectomy, unloading, and depression [10, 16, 17]. Conversely, β-AR stimulation reduced bone mass in mice and rats [18–20]. In line with these findings, mice lacking β2AR or adenylyl cyclase 5, a downstream mediator of β2AR signaling pathways, showed high bone mass phenotypes and protective effects against age-related bone loss, respectively [15, 21]. Importantly, conditional knockout of osteoblast β2AR was associated with increased bone mass in mice through stimulated bone formation and reduced bone resorption [22], as seen also in global β2AR-deficient mice [15]. These observations indicate that particularly the β2AR in osteoblasts is crucial for sympathetic neural effects on skeletal functioning in rodents.

Molecular studies have clarified in more detail the mechanisms by which sympathetic signaling in osteoblasts controls bone formation and bone resorption. Fu et al. [23] showed that osteoblasts express peripheral clock genes such as *Per1* and *Per2* and that sympathetic neural stimulation of β2AR pathways in osteoblasts increases expression of these circadian genes via cAMP response element binding protein (CREB), which downregulates transcription of *c-myc* and its target, *cyclin D1*. A series of these processes in osteoblasts might contribute to the antiproliferative effects of SNS activity that act to reduce bone formation. Although the β2AR is known to be expressed in osteoclasts as well [11, 24], studies using β2AR-deficient mice indicate that the SNS may indirectly affect bone resorption by affecting osteoblasts [15]. Activation of β2AR in osteoblasts by sympathetic signals stimulates protein kinase A-induced phosphorylation of ATF, a cell-specific CREB-related transcription factor that is essential for osteoblast biology [25]. This directly stimulates the production, by osteoblasts, of the osteoclastogenic factor, receptor activator of nuclear factor-κB ligand, leading to increased bone resorption [25].

Collectively, these animal studies provide robust evidence that the SNS negatively regulates bone remodeling by simultaneous inhibition of bone formation and stimulation of bone resorption, primarily through β2AR pathways in osteoblasts.

Clinical Pharmacological Observations

Prompted by the increasingly advanced understanding of SNS effects on rodent skeletal functioning, numerous epidemiological studies have been performed to assess the role of the SNS in human bone metabolism. Because of the invasiveness of direct measurement methods of sympathetic output in humans, e.g., intra-arterial catheterization and microneurography of the peroneal nerve [26, 27], the majority of clinical studies has been performed of β-AR modulators.

Selective and nonselective β-blockers have long been used as major antihypertensive medications Several observational studies found that β-blocker treatment is associated with increased bone mass and/or reduced fracture risk [28–31], in agreement with the rodent data described above. Furthermore, a recent meta-analysis of 16 case–control and cohort studies showed that the risk of any fracture may be about 15% lower in subjects receiving β-blockers than in controls [32], potentially indicating a protective effect of β-blockers on human bone health. However, results from other studies failed to support this notion. Longitudinal studies did not find any relationship between the use of β-blockers and the occurrence of osteoporosis-related phenotypes [33–35]. In addition, polymorphisms of the β2AR, which might perturb its

function, were not associated with changes in fracture risk or bone mineral density (BMD) [36]. More importantly, two prospective, randomized pharmacological intervention studies showed that β-blockers do not affect human bone turnover [37, 38]. Some reports even showed an increased fracture risk in patients treated with β-blockers [39, 40]. For example, analysis of nationwide claim data in South Korea, covering 8,315,709 subjects with antihypertensive prescriptions, showed that, after adjustment for potential confounding variables, β-blocker users had a 15% higher risk of fractures than nonusers [39]. Thus, the impact of a β-adrenergic pharmacological blockade on human bone health is a long-standing debate.

Clinical studies of the association between β-agonists and bone health are limited and do not provide convincing evidence supporting a pivotal role of SNS on human bone remodeling either. In three cross-sectional studies of patients with chronic obstructive pulmonary diseases, prescription of β-AR agonists was not associated with fracture risk [41–43], and a prospective study showed that selective β2AR agonists do not affect the levels of bone formation and resorption markers in postmenopausal women [37]. The overall inconclusive results from the studies using β-blockers or β-agonists might be attributable to significant heterogeneity of the examined populations, AR selectivity, different dosages and durations of therapy, co-morbidities, and interactions with other medications. Therefore, an adequate answer to the question of whether bone metabolism in humans is regulated by the SNS would require a human disease model simulating sympathetic activity.

Lessons from Pheochromocytoma

Pheochromocytoma is a rare neuroendocrine tumor arising from the chromaffin cells of the adrenal medulla and is characterized by an excessive production of catecholamines [44]. The hallmarks of catecholamine secretion, such as headache, palpitation/tachycardia, and sweating, are commonly mentioned as a triad of symptoms that suggest pheochromocytoma [45]. Considering that catecholamines are the key mediators of SNS effects on peripheral tissues [5], pheochromocytoma might be an ideal human model to determine the importance of sympathetic activity on the pathogenesis of a variety of diseases. This concept has inspired efforts to investigate skeletal changes in patients with pheochromocytoma.

In a case–control study that included patients with ($n = 21$) and without ($n = 126$) pheochromocytoma, Veldhuis-Vlug et al. [46] found that plasma levels of C-terminal telopeptide of type 1 collagen (CTX), a bone resorption marker, were significantly higher in cases than in controls; importantly, these elevated levels normalized after adrenalectomy. However, plasma levels of procollagen type 1 N-terminal propeptide, a bone formation marker, did not differ between the two groups, nor did they change after adrenalectomy. Despite its lack of an assessment of bone mass, this study is meaningful in that it drew attention of clinicians to the potential role of the SNS in human bone remodeling. As an extension of this, recent study investigated the effects of catecholamine excess on BMD and bone turnover rate in 31 patients with histologically confirmed pheochromocytoma, as well as in 280 patients with nonfunctional adrenal incidentaloma, defined as cases and controls, respectively [47]. After adjustment for confounding variables, urinary levels of metanephrine and normetanephrine—i.e., the respective O-methylated metabolites of epinephrine and norepinephrine—were positively correlated to serum levels of CTX, but not to those of the bone formation marker, bone-specific alkaline phosphatase (BSALP). Furthermore, patients with pheochromocytoma had 7.2% lower lumbar spine BMD and 33.5% higher serum CTX levels than controls, although no significant between-group differences were found in serum BSALP. Finally, the odds ratio for lower BMD at the lumbar spine was 3.31-fold higher in patient with pheochromocytoma than in those without. Together, these findings provide clinical evidence that catecholamine excess and the concomitant sympathetic overstimulation in pheochromocytoma may contribute to decreased bone mass as well as increases in bone resorption.

In a healthy adult skeleton, the balance between bone resorption and formation is tightly regulated in a local, coordinated, and sequential manner, which is referred to as the "coupling phenomenon" [48, 49], and abnormalities in these two processes lead to disorders of bone metabolism [3]. The studies by the Veldhuis-Vlug [46] and Kim [47] groups showed increased bone resorption with unaltered bone formation rates in patients with pheochromocytoma. These results indicate that the subsequent bone loss by sympathetic overstimulation in human bone remodeling could be explained as an uncoupling phenomenon, characterized by excessive bone degradation, rather than by reduced bone formation. This pattern of uncoupling in humans is quite different from that in rodents, in which both osteoblasts and osteoclasts are targeted by sympathetic signals [15, 22]; also, this discrepancy is consistent with the notion that murine experiments cannot fully recapitulate the actual mechanisms in the human body. Although the exact mechanisms behind the disparate responses of SNS to bone remodeling between humans and rodents remain unclear, existing evidence supports that a key factor may lie in the tighter SNS control of osteoclast biology in humans. Hence, it could be speculated that antiresorptive agents might provide a more effective therapeutic option to maintain bone health than bone-forming drugs in clinical cases with increased sympathetic activity.

Although BMD measurement by dual-energy X-ray absorptiometry (DXA) is among the best available method to

determine osteoporotic fracture risk, approximately two thirds of individuals who are suffering from a bone fracture do not have BMD-defined osteoporosis [50]. These findings suggest that the BMD test alone is not sufficient to adequately assess bone quality, resulting in insufficient evaluation of bone strength. The trabecular bone score (TBS), which is generated by quantifying pixel gray-level variations on lumbar spine DXA scans, has been introduced recently as a parameter representing bone microarchitecture, a key determinant of bone quality [51, 52]. Following numerous epidemiologic studies demonstrating that low TBS values predict osteoporotic fractures regardless of BMD [53–55], TBS analysis is now considered to be a clinically feasible noninvasive tool for the assessment of bone properties that cannot be captured by standard BMD measurement [56]. To clarify the role of SNS-related effects on human skeletal deterioration, recent study employed this valuable method [57]. In a Korean cohort consisting of patients with pheochromocytoma ($n = 29$) and controls ($n = 266$), subjects with pheochromocytoma had, on average, 2.9% lower lumbar spine TBS values than those without pheochromocytoma; additionally, urinary normetanephrine levels were inversely correlated with lumbar spine TBS values. Furthermore, subjects in the highest urinary normetanephrine quartile showed markedly lower lumbar spine TBS than those in the lowest quartile. These results suggest that in humans, excessive sympathetic activity, besides stimulating bone resorption and accelerating bone loss, may also contribute to bone fragility by weakening bone microarchitecture.

The spine is primarily composed of spongy, cancellous bone, with a so-called trabecular architecture, whereas long bones, including the proximal femur, are composed primarily of cortical bone. The most notable findings from studies of bone phenotype in pheochromocytoma patients were a markedly reduced bone mass of the lumbar spine—albeit not of the femoral neck and total hip—and an increased risk of low BMD only of the lumbar spine [47]. Furthermore, as described above, sympathetic overstimulation in pheochromocytoma was associated with significantly lower values of lumbar spine TBS [57]. These results suggest that the effects of SNS activity might be more dominant in trabecular bone rather than in cortical bone. This hypothesis is supported by a human study, in which skeletal microstructure was measured using high-resolution peripheral quantitative computed tomography in premenopausal and postmenopausal women [58]. In this study, sympathetic activity correlated negatively with trabecular bone volume, thickness, and connectivity density and positively with trabecular separation, whereas no significant relationships were found between cortical bone parameters—such as cortical volumetric BMD, cortical thickness, endocortical circumference, periosteal circumference, and cortical pore volume—and sympathetic activity. Several possible mechanisms may explain the different effects of SNS activity according to skeletal type. First, Farr et al. [58] hypothesized that skeletal responses to sympathetic stimuli may be heterogeneous because of different patterns of innervation of the various skeletal regions by sympathetic nerve fibers. Second, trabecular compartments are known to be more metabolically active than cortical compartment due to their greater surface area; thus, the skeletal regions such as the lumbar spine, which are primarily composed of cancellous bone, might be more vulnerable to the detrimental effects of sympathetic overstimulation. Third, it is possible that mechanical loading of bones such as the femur or tibia could counteract the adverse effects of sympathetic signals [59]. Because all of these mechanisms are still hypothetical, further studies are necessary to provide definite confirmation in humans.

Specific Subtypes of AR Mediating Sympathetic Activity on Human Skeletal Physiology

Observations in pheochromocytoma patients clearly indicate the importance of sympathetic outflow in human bone metabolism; however, they provide no information about the specific subtypes of AR mediating these effects. Bone cells, including osteoblasts, osteoclasts, and osteocytes, express not only β2AR but also, to a lesser extent, β1AR, β3AR, α1AR, and α2AR [60–64]. Therefore, it is overall possible that in humans, AR pathways other than those activated by the β2 receptor with its predominant role in rodent skeleton [10, 15, 22] are partially responsible for the SNS regulation of bone remodeling. In fact, a recent clinical study demonstrated that postmenopausal female patients receiving β1-selective blockers possess a better bone microarchitecture than patients who do not and that treatment with β1-selective blockers (atenolol and nebivolol), but not a nonselective β-AR blocker (propranolol), reduces serum CTX levels and increased BMD of the ultradistal radius, relative to placebo [65]. The authors of this study concluded that bone metabolism in humans might be predominantly regulated by β1AR-related signals, rather than by β2AR-related signals. In addition, because bone is a highly vascularized tissue, α1AR activation targeting smooth muscle layers around vessels [66] might result in the adverse skeletal outcomes via vasoconstriction and reduced blood flow. Further research studies focusing on specific receptor subtypes are necessary to gain a complete understanding of the exact neuronal mechanisms by which the SNS controls human skeletal physiology.

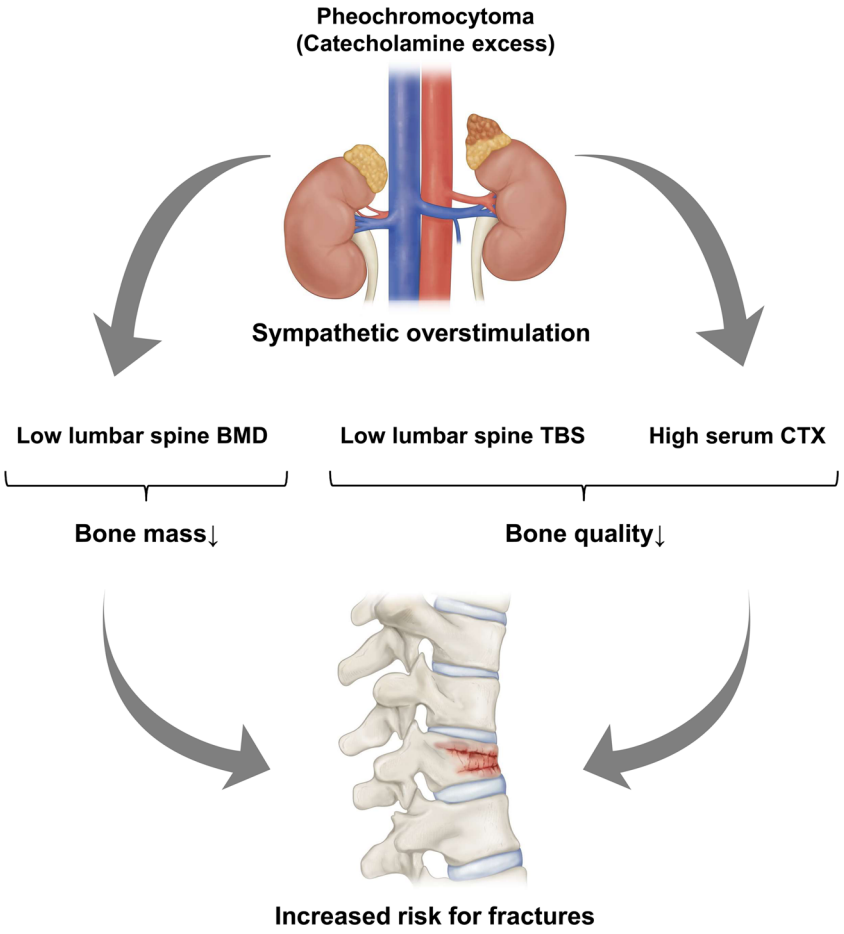

Fig. 1 Catecholamine excess and the resultant sympathetic overstimulation in pheochromocytoma can contribute to weakened bone strength, especially in trabecular bone, by adversely affecting not only bone mass but also bone quality in humans. BMD bone mineral density, TBS trabecular bone score, CTX C-terminal telopeptide of type 1 collagen

Conclusion

Fragility fractures associated with high morbidity and mortality result from reduced bone strength, which is determined not only by bone mass but also by bone quality-related factors such as bone turnover and microarchitecture. In fact, both low BMD and high bone resorption rate—as static and dynamic indicators, respectively—are important predictors of fractures [67, 68]; when these conditions occur simultaneously, risk of fractures is markedly increased [69]. Lower values of lumbar spine TBS, which are indicative of skeletal deterioration, are also known to be associated with a significant probability of fracture, either independently of or synergistically with BMD [53–55]. Although clinical studies addressing osteoporotic fracture as a primary endpoint in pheochromocytoma have not been performed because of the rare nature of this disease, existing evidence from a human disease model that encompasses SNS effects indicates that in humans, catecholamine excess and sympathetic hyperactivity can lead to a weakening of bone strength; this is especially the case for trabecular bone, because of deleterious effects on bone quality as well as bone mass (Fig. 1). If the reversibility of deteriorated bone parameters observed in pheochromocytoma is demonstrated by adrenalectomy in future studies, pheochromcytoma could be regarded as a potential risk factor for osteoporosis and related fractures.

Apart from phrochromocytoma, a rare neuroendocrine tumor, there are several common clinical circumstances that involve both increased sympathetic signaling and skeletal abnormalities, such as aging [70], postmenopause [58], severe depression [17, 71], or unloading [16, 72]. On the basis of accumulating data demonstrating detrimental effects of sympathetic hyperactivity on human bone remodeling, clinicians might expect that pharmacological blockade of sympathetic pathways might provide a viable antiosteoporotic therapeutic option to improve bone health under these conditions. However, there is lack of clinically relevant, practical information on the specific type and exact dosage of AR modulators that would be needed to exert beneficial effects on the human skeleton. Considering that one of the major objectives of bone research is to reduce the burden related to fragility fracture, which is associated with high disability and morbidity, it is now time to perform conclusive randomized and controlled trials to quantify the effects of available AR interventions, using fracture as a clinical endpoint.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects performed by any of the authors.

Informed Consent This is not needed as no studies on human subjects were performed.

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