

Sympathetic nervous system and bone adaptive response to its mechanical environment

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Abstract

While bone adaptive response to its mechanical environment was considered to be controlled locally by cytokines and systemic hormones, some recent work suggests that it could also be neurally regulated. Bone is indeed very densely innervated and many experimental and clinical studies have previously shown the involvement of the nervous system in the control of bone metabolism. The demonstration that the central nervous system regulates bone mass via the sympathetic nervous system (SNS) has prompted recent studies aimed to investigate the role of the SNS in the bone mechano-adaptive response. This review will focus on this work and summarize the evidence for a contribution of the β -adrenergic signalling in the response of bone cells to mechanical loading. The apparent conflicting results obtained in diverse experimental models of loading and unloading, at different skeletal sites, and in relation to various hormonal levels, will be discussed. While those studies do not support a major influence of the SNS on the bone mechano-adaptive response, there is nevertheless strong evidence that the SNS is part of a complex system which contributes to the metabolic regulation of bone.

Keywords: SNS, β -adrenergic Signalling, Propranolol, Bone, Mechanical Loading

Introduction

Bone is a dynamic tissue that can adapt its mass and architecture to be constantly structurally suitable to the external mechanical demand. To achieve this, bone cells can enhance bone formation with increasing mechanical demands or dispose of bone in excess in situations of disuse. This bone adaptive response to the strain magnitude, originating from the external environment, defines the mechanostat, a concept postulated by Frost¹. According to this theory, there is a threshold called "the minimum effective strain" which is the lower strain within the remodelling window of strains under which bone resorption prevails over formation. On the reverse, strains above the upper threshold of the remodelling window will increase formation over resorption. The physiological mechanisms by which a mechanical stimulus is sensed

by bone and by which the sensed signal is transduced into biochemical signals by bone cells are not completely understood. Bone cells respond to intermittent, but not to static loading, implying a sophisticated perception system². They detect changes in their strain environment associated with fluid flow and physical deformation. Osteocytes, with their unique location and morphologies, and osteoblasts on the bone surface are believed to be the mechanosensors of bone. They communicate via gap junctions providing a network important for both mechanosensation and mechanotransduction. Within minutes of a mechanical stimulus, those cells release prostaglandins and nitric oxide which respectively enhance bone formation and inhibits bone resorption^{3,4}. While this bone adaptive response to its mechanical environment was considered to be controlled locally by growth factors, cytokines and systemic hormones⁴, several studies suggest that it could also be neurally regulated.

Both bone and periosteum are richly innervated⁵ and the areas of mineralized bone which receive the greatest mechanical load display the highest density of nerve fibres⁶. An ancient hypothesis was that nerve endings could be the receptors of mechanical stress in bone^{7,8}. This is supported by a recent study indicating that load by occlusal force causes an increase in the number of nerve fibers around oral implants⁹. Hert et al. in 1971⁸, have however demonstrated

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that innervated and denervated limbs react to loading in the same way, and several groups have shown that bone explants and isolated bone cells are sensitive to mechanical stimuli^{10,11}, opposing this assumption. Nevertheless, the idea that the nervous system, and particularly the sympathetic nervous system (SNS), might contribute to the bone adaptive response mechanical loading has emerged after the demonstration that the central nervous system regulates bone formation and bone mass via the SNS acting on β 2-adrenergic receptors expressed by osteoblasts¹². This discovery that neuronal signals make a major contribution to bone mass regulation has been one of the most exciting developments in the field of bone metabolism research in recent years. Although it had a major impact, the idea that the nervous system influences bone remodelling is not new and many experimental and clinical studies have previously shown the involvement of the nervous system in the control of bone metabolism^(for reviews, see 13-18). Both sensory and sympathetic nerve fibers are present in bone and bone cells express a variety of receptors for neuromediators, including β -noradrenergic receptors^{5,12}. It is still unclear whether the major influence of the nervous system on bone physiology is local via peripheral skeleton innervation or central. It is likely that both central and peripheral controls occur. Direct neural regulation of bone cells via paracrine release of neuromediators by nerve terminals is expected, but there is also increasing evidence that the hypothalamus senses the physical and metabolic needs of the skeleton, and integrates those needs with other homeostatic functions, to control bone metabolism^{12,19}. Whether the central nervous system regulates bone cells' activities only via peripheral innervation or also via a soluble factor is unknown. Moreover, neural influences may be exerted on bone cells indirectly via the control of blood supply and immune cell functions²⁰⁻²². The signal transmission from nerve to bone cells is also a subject of a debate as no synapse was identified in bone⁵. It is expected that non-synaptic secretion of neuromediators and neuropeptides occurs, and that GAP junctions between osteoblasts and osteocytes could be involved in the transmission of neuronal signals^{5,17,18}. The link between osteocytes, the main sensors of mechanical loading, and bone innervation is unclear. The opportunity for osteocytes to come in direct contact with innervation is limited, but neuromediators may diffuse through the lacunocanalicular system. There is evidence that osteocytes express receptors for neuromediators and that the expression of some of these receptors is modulated by mechanical loading²³⁻²⁶.

A potential role of the SNS in the adaptation of bone to mechanical signals was suggested by several studies. The first indication came from reports in bedridden patients and astronauts under gravity conditions. Those people lose bone due to the deficit of mechanical stress but the mechanisms underlying such disuse osteoporosis are mostly unknown. The rapid bone loss in unloading conditions could evoke the involvement of the nervous system which can elicit very rapid signals. The well-known effect of exercise on the SNS activa-

tion²⁷, together with the fact that the sympathetic nervous tone was enhanced in the astronauts' muscles after returning from space²⁸, suggested a possible role of the SNS. β -adrenergic agonists have been widely used as anabolising agents on skeletal muscles^{29,30}, and studies on hormonal responses to exercise have shown that exercise and growth hormone release are coupled to adrenergic activation³¹. More recent studies in rats and mice have supported the involvement of sympathetic tone in the induction of bone resorption after unloading. They indicate for the first time that the β -adrenergic part of the SNS is a mediator of the physiologic response to skeletal unloading, as treatment with β -blocker propranolol suppressed the unloading-induced reduction in bone mass^{32,33}. Those results obtained in the hind limb unloading model of tail suspension have however not been confirmed in the most widely used model for disuse, the sciatic neurectomy model³⁴. Other studies strongly suggest that the adrenergic component of the SNS is not involved in the bone osteogenic response to mechanical loading³⁵, although the observation that mechanical loading of one region of a long bone induces an osteogenic response in a distant skeletal site at which bone strain is not affected by loading³⁶ may nonetheless evoke the involvement of the nervous system. The evidence for a potential role of the SNS in the bone mechano-adaptive response therefore remains weak. Clinical studies investigating the effects of β -blockers on bone mass have not simplified the interpretation of all these experimental data as they have shown conflicting outcomes. Furthermore, there are no obvious common signalling pathways between adrenergic receptor signalling and the mechanical pathway, although both pathways lead to an increase in the production of prostaglandins E2 in osteoblasts^{4,37}. This review will focus on the complexity of this neuronal system and its role on the bone mechano-adaptive response, and will try to clarify those apparent differing results.

The SNS does not influence bone functional adaptation to mechanical loading

Traditionally, bone adaptation to mechanical loading has been considered highly site-specific³⁸ and not centrally controlled⁸⁻¹⁰. Consistently, using a model for non-invasive *in vivo* axial loading of murine tibia previously developed in our group³⁹, we showed that the cortical bone gain induced by cyclic loading of tibiae was not modulated by the SNS³⁴. In this set of loading experiments, the SNS was inhibited by either a high dose of the non-selective β -adrenergic receptor antagonist propranolol (PRO, 0.5 g/liter in the drinking water) or guanethidine sulphate (GS, 40 mg/kg/day), a treatment that reduces norepinephrine concentration in the peripheral SNS. New cortical bone formation was enhanced by loading in all tibial sites examined and the increases in new bone formation induced in response to mechanical loading were similar in mice treated with either GS or PRO compared to controls, indicating that inactivation of the SNS

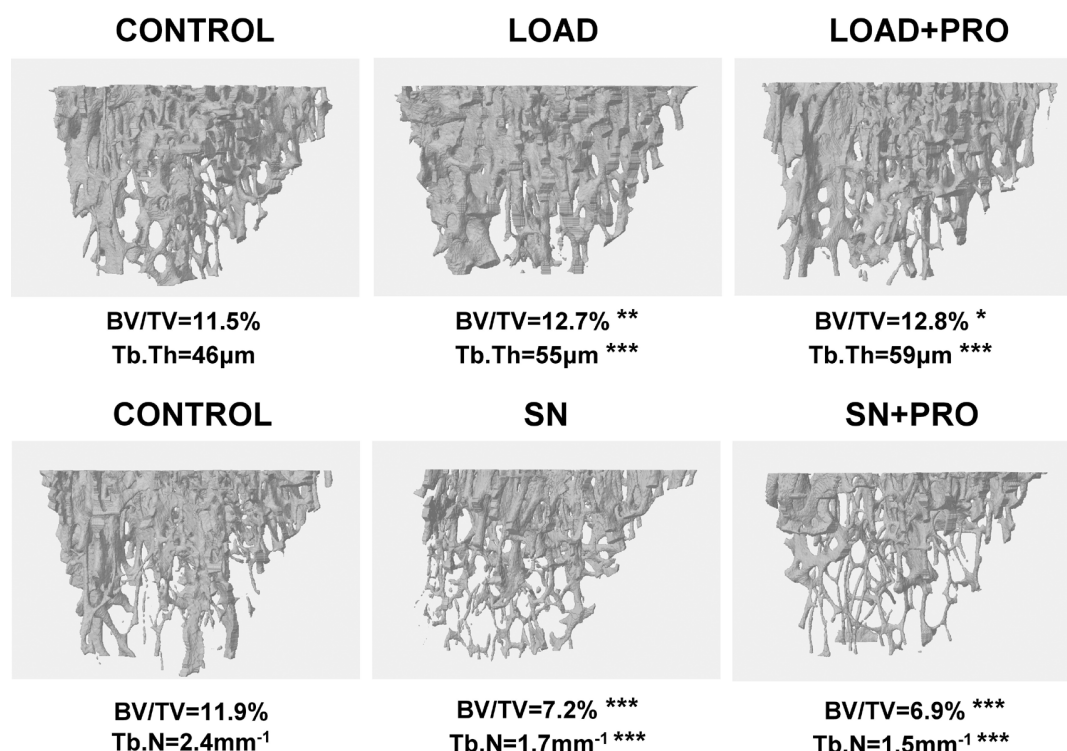


Figure 1. Representative three-dimensional reconstructions of the trabecular bone within the tibia metaphysis. External loading (LOAD) of the tibia increased microarchitectural parameters. Unilateral Sciatic Neurectomy (SN) decreased bone mass and structure. Treatment with propranolol (PRO) did not modulate either the anabolic or the catabolic bone response to the mechanical environment. Data reproduced from Marenzana et al.³⁴ Statistics: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control tibia determined by 2-way ANOVA and Bonferroni's post hoc analysis.

had no effect on load-induced cortical new bone formation.

However, parallel studies have shown a contribution of the SNS in the bone loss induced by hind limb unloading. In this model, load bearing on the hind limbs is prevented, greatly reducing the strain normally generated during cage activity (sub-physiological strain level). Thus, this model represents the other end of the mechanical spectrum applied to bone in comparison to the tibia axial loading model mentioned above which induces supra-physiological strain levels. Treatment of rats and mice with similar high concentrations of β -blocker PRO and/or GS, prevented the trabecular bone loss induced by hind limb unloading in the tail suspension model^{32,33}. Analyses on the cellular bases showed that the SNS mediates the unloading-induced bone loss through suppression of bone formation by osteoblasts and enhancement of resorption by osteoclasts. In those two studies however, the first one did not examine the cortical bone response to unloading³³, while the second one³² did not demonstrate a significant cortical bone mass recovery induced by PRO treatment in the tail-suspended group. This suggested that the SNS modulation of unloading-induced bone loss is more prominent in the cancellous bone compartment. One hypothesis made following those observations was that the

cortical bone response to loading could be mediated primarily by hormonal factors responsible for the anabolism of cortical bone such as estrogen status and insulin levels, whereas neuronal influences may control predominantly trabecular bone mass. This skeletal site-specific bone mass regulation is supported by a study showing that the phenotypic effects of leptin deficiency differ between the long bones and the vertebrae⁴⁰. This hypothesis was nevertheless disproved in a subsequent study performed by our group³⁴ which demonstrated that the mechano-adaptive response of trabecular bone in the tibia metaphysis was also not affected by blockade of the β -adrenergic receptors using PRO. This held true in conditions of bone loss caused by sub-physiological – near zero – loading in the sciatic neurectomy model as well as in conditions of bone gain induced by supra-physiological external mechanical loading (Figure 1). Thus, these findings strongly suggest that the sympathetic tone is not involved in the modulation of the local bone response to its mechanical environment. As a consequence, the abundant nerve fibers distributed over the periosteum as well as on the trabecular surfaces⁵ do not seem to act as mechano-receptors or mechano-transmitters for the mechanical loading in bone¹¹. On the contrary, trabecular and cortical bone compartments

appear to have consistent responses to loading which are not modulated by the SNS, thus excluding that differences in the density of sympathetic innervation or changes in the expression of adrenergic receptors between trabecular and cortical bone could play a role in the contrasting responses to mechanical loading observed previously in trabecular and cortical bone. Consistently, the catabolic action of β -agonists on bone^{41,42} was not alleviated by treadmill exercise which supplies a potent local remodelling stimulus to the long bones. It combines direct bone straining during the exercise with enduring mechanical stimulation from enhanced muscle mass which is also further increased by the action of the β -agonists⁴³. It is worth taking notice of the differences between the treadmill-based model of exercise and the tibia axial compression model. While in the latter the cyclic compression is administered under complete muscle relaxation (general anaesthesia), the exercise model implies up to 70% maximal O₂ consumption⁴⁴ involving changes in muscle mass, heart beat rate, blood pressure and even hormone levels, such as leptin⁴⁵, besides the changes in bone. Therefore, the model for applying axial loads is more suitable to identify the modulation of bone adaptation to loading independently of other systemic factors, while running exercise takes into account all the physiological changes in a more complex system in which the direct modulation of the bone mechanoadaptive response is less clear to identify.

Contrasting effects of the SNS on the bone loss induced by hind-limb unloading and sciatic neurectomy

The divergent influence of the SNS on the bone loss induced by two different models of disuse, the hind limb unloading and sciatic neurectomy, remains unclear. In both models, the same β -blockade (high dose propranolol, 20 mg/ml) and a similar timeframe of two weeks of unloading were used. Several factors may contribute to those differences. First, there is differential impairment of the bone remodelling homeostasis in these two models, with bone resorption being more markedly increased by neurectomy^{46,47} and to a lesser extent by tail suspension⁴⁸, while in the latter model bone formation is highly suppressed⁴⁹, which might have been targeted preferentially by the SNS blockade. Second, different hind limb muscles are affected by unloading in these two models, although it is unclear how this is related to the greatest effects of the SNS on bone mechanical properties induced by tail suspension compared to neurectomy⁵⁰. Third, the tail suspension model is known to involve several physiological shifts, such as alterations in the SNS activity and in neuromuscular function similar to that observed after spaceflights²⁸, changes in blood distribution with reductions in plasma volume, perturbations in the arterial vascular tone^{51,52}, and decrease in femoral intramedullary pressure⁵³. In addition, stress-related factors may also play a role in the tail suspension-induced bone loss

besides mechanical unloading, which fits with the recent finding demonstrating that stress-induced depression induces bone loss in mice through stimulation of SNS⁵⁴. It is also possible that single housing of rodents, as it is observed in practice in the tail suspension model, might have an impact on bone, analogous to what has been shown in space-flight⁵⁵ in which the housing conditions, isolation versus social, dramatically influenced bone response. Finally, the sciatic neurectomy model involves significant reduction, although not complete deletion, of the innervation in the neurectomized limbs⁵⁶. As the sciatic nerve is a mixed nerve, which contains both sympathetic and sensory nerve fibers⁵, it is possible that there is a decreased normal nerve sympathetic transmission in the rat tibia after sciatic neurectomy and/or that other components of the nervous system are activated by or counterbalanced by the β -blockade of the adrenergic signalling pathway. Thus, alternative experimental models of immobilization in rodents, which do not induce significant physiological changes such as the tail suspension, or do not affect the normal neural transmission to/from the limbs such as neurectomy, are needed in order to dissect the true effect of SNS blockade on the bone loss induced by unloading. The possible alternative models, known to induce osteopenia, include casting⁵⁷ or muscular disconnection through tenotomy⁵⁸, although tenotomy was found difficult to apply in mice (C. Chenu, unpublished observations). Another animal model of disuse in which innervation remains intact is the MyoD-Myf5-deficient mice which lack skeletal muscle, but the use of those mice is limited by the fact that they die soon after birth⁵⁹. A possible way to clarify this contrasting influence of the SNS in these two models of disuse would be to test the protection against unloading-induced bone loss in these two models in mice deficient for β 2-adrenergic receptor.

Finally, the choice of propranolol and the high doses used in those studies are critical. The exact effects of PRO on bone are complex and difficult to dissect since they may vary depending on the dose used. Furthermore, the dose-effect may differ according to the bone site. As demonstrated for other cells⁶⁰, propranolol depending on the dosage might produce paradoxical effects on bone cells, simultaneously reducing cAMP accumulation by acting as an inverse agonist while working as an agonist on MAP kinase activation. Most effects on bone of β -adrenergic signalling seem to be mediated by β 2-adrenoreceptors expressed by bone cells¹². However, expression of other β -adrenergic receptors (ADRB) subtypes have been suggested in bone and bone marrow⁶¹ (and personal unpublished results), and we cannot completely exclude the possibility that they may also contribute to the regulation of bone mass by having different or even opposite effects on bone. This is suggested by the phenotype of ADRB1/B2 double KO mice which shows a very different phenotype of ADRB2 KO, illustrated by the reduced trabecular and cortical thickness⁶². The author of this study suggested in a review that stimulation of β 2-adrenergic receptors on osteoblasts leads to bone loss via RankL-

mediated osteoclastogenesis, while activation of $\beta 1$ -adrenergic receptors may contribute to maintain cortical bone mass by affecting the GH-IGF-1 anabolic pathway⁶³. Therefore, it is possible that propranolol unspecific blockade of all three ADRB subtypes might result in various opposite effects on bone mediated by different adrenoreceptors or subtypes. How these possible effects might be related to the changes in bone mass in these two different unloading models is currently unknown.

Studies on the effects of β -agonists on bone do not help to clarify the discordant effects of ADRB blockade in these two different unloading models. Clenbuterol, a $\beta 2$ -agonist, has been shown to reduce the bone loss induced by both hind limb unloading via tail suspension⁶⁴ and denervation⁶⁵, which appeared to be correlated to a decrease in muscle wasting. Conversely, the extent of the catabolic effects of β -agonists such as isoproterenol, clenbuterol or salbutamol, established on control rodents^{41,42,66,67}, appears to be reduced in the presence of pre-existing extensive bone loss, as observed in OVX rats⁶⁸ or hind limb unloaded mice³³. This catabolic action of β -agonists persisted in the OVX rats if they were subjected to exercise, thus abolishing the positive effect on bone of the additional mechanical loading stimulus despite the concomitant anabolic effects of these compounds on the hind limb muscle mass. Although the absence of estrogen limited the influence of β -agonists on bone, the deleterious skeletal effects of β -agonists may also depend on the initial structural bone quantity and quality⁶⁸.

Changes in hormonal levels may affect the influence of the SNS on bone mechanoadaptation

The interactions between estrogen, mechanical loading, and the β -adrenergic axis have been recently demonstrated by a series of publications by Bonnet and colleagues⁶⁸⁻⁷⁰. They first demonstrated that only low doses of propranolol are beneficial for preserving trabecular bone mass in ovariectomized rats. These results are in agreement with earlier findings showing that rats with bone defects treated with low doses of PRO have increased callus formation and bone union⁷¹. The protective effect of PRO on the bone loss induced by OVX in mice remains however controversial as PRO was reported to either prevent bone loss⁶⁶ or to be ineffective unless combined with PTH treatment⁷². In both of those studies PRO was given at a high dose in the drinking water. It is therefore possible, as previously discussed, that high doses of PRO might have an inverse agonist effect on β -AR which could be detrimental to the SNS modulation of bone loss⁷⁰. This hypothesis is supported by the observation of the bone phenotypes of β -adrenergic transgenic mice. While $\beta 2$ -AR deficient mice have a high bone mass phenotype and are resistant to ovariectomy¹², $\beta 1/\beta 2$ -AR double deficient-mice present a low bone mass phenotype and are not resistant to OVX^{62,73}. The interaction among these receptors may also play a role since the deletion of $\beta 1$ -AR solely does not yield any bone phenotype¹², while the triple

deletion of all β -ARs generates a high bone mass phenotype and the mice are not protected from OVX-induced bone loss⁷⁴. Interestingly, the fact that high doses of PRO can be effective in rescuing the bone loss induced by OVX, while it has no effect on the bone deficit induced by sciatic neurectomy, suggests that the differences in trabecular microarchitecture and cortical modelling observed in these two osteopenic models⁷⁵ might be mediated by different catabolic signalling pathways, with only the estrogen signalling axis being modulated by the SNS.

Bonnet et al.⁶⁹ also showed an additive effect of low doses of PRO and exercise on cortical porosity and overall bone mechanical strength in OVX rats compared to the effects of PRO and exercise alone in those rats, although no combined effects were observed on trabecular microarchitecture. In contrast, PRO inhibited the effect of exercise and exercise inhibited the effect of PRO on trabecular bone, suggesting that the SNS is involved in the trabecular response to exercise in the absence of estrogen but not the cortical response. However, the absence of synergistic effect of mechanical loading with propranolol treatment in estrogen-intact animals³⁴ strongly suggests that the SNS modulation of bone response to its mechanical environment is activated primarily in the presence of a hormonal imbalance. This view is also supported by the finding that hind limb suspended rats, whose bone loss has been shown to be modulated by SNS blockade³³, have significant decreased serum leptin levels, while exogenous leptin administered peripherally restores their bone mass⁷⁶. Leptin regulation of bone mass and its connection to the SNS is complex as leptin can have both direct anabolic effects on bone formation and multifaceted central effects including the stimulation of the GH-IGF-1 axis, the suppression of neuropeptide Y a potent inhibitor of bone formation, and an increase in trabecular bone remodelling mediated by the SNS⁶³. It seems presently unclear how the decrease in peripheral leptin in the hind limb suspension model could be linked to the rescuing effect of the SNS blockade on the unloading-induced bone loss. Nonetheless, those observations further support the suggestion that the SNS influence on bone remodelling may be dependent on hormonal changes such as estrogen deficiency and decreased serum leptin levels.

Unsolved questions remain concerning the high bone mass phenotype achieved by the transgenic mice lacking $\beta 2$ -AR. Indeed, this phenotype is acquired in the presence of estrogen and those mice are protected from OVX-induced bone loss¹². One may question whether the deletion of $\beta 2$ -AR combined with the exercise and the normal mechanical loading experienced by the mice in their cages, results in higher bone mass compared to wild type littermates. The answer to this question would need not yet available data regarding bone responses to external mechanical loading and unloading of these transgenic mice compared to wild type mice. However, the fact that bone mass was increased equally in both appendicular (heavily subjected to load bearing) and axial (less load bearing) skeleton might be an indi-

cation that deletion of $\beta 2$ -AR induces enhanced bone formation systemically, irrespectively of the local mechanical stimuli. Generally, the interpretation of transgenic models in relation to their adaptive response to mechanical loading is also complicated by the fact that the deletion is present from birth, and therefore the acquisition of the phenotype might involve the modulation of the fast growth phase rather than the subsequent slower modelling/remodelling process. The demonstration that high dose propranolol has a protective effect on bone of young OVX mice (6-weeks-old)⁶⁶, but not of adult OVX mice (15-weeks-old)⁷² and rats (6-months-old)⁷⁰, might suggest that β -ARs signalling could interact preferentially with the fast growing phase in rodents, but this needs to be investigated.

Clinical studies

Several clinical retrospective studies have investigated the use of β -blockers as potential therapeutic options for osteoporosis⁷⁷⁻⁸⁵. Those studies have revealed conflicting results, although they generally showed a positive correlation between the use of β -blockers and bone mineral density. The β -blockers used in those studies were mainly $\beta 1$ -selective or nonselective, indicating that the protective effects of those β -blockers on bone mass might be mediated via sympathetic blockade of $\beta 1$ -adrenoreceptors⁸⁶. This argues against the view that all effects of the SNS on bone are mediated by actions on $\beta 2$ -receptors expressed by osteoblasts⁶⁶. Those clinical data mean that the effects of β -blockers on bone in humans are complex and involve different β -adrenergic signalling pathways. The relationship with exercise was not often investigated in those studies. When physical activity was documented, the correlation between β -blocker use and BMD was independent of the correction for physical activity^{81,84}, further supporting the view that the SNS is not involved in the local bone mechanotransduction. Interestingly, in the last cross-sectional study performed by Bonnet et al.⁸⁴ on postmenopausal women, the positive effect of β -blockers on BMD was paralleled by a positive effect on the trabecular architecture in the calcaneus, which is arguably one of the most sensitive bone sites in regards to mechanical loading induced by physical activity. Correction for physical activity in this cohort of patients again did not influence the effect of β -blockers on trabecular bone microarchitecture.

Perspectives

The overview of animal studies and clinical data using β -adrenergic receptors antagonists and agonists points towards the exclusion of a direct modulation of the SNS on bone modelling/remodelling in response to the mechanical environment. There are presently no data supporting a role for the SNS in the regulation of load-induced bone formation, indicating that other mechanotransduction pathways regulate bone formation in loaded bones. Most reports also

exclude a contribution of the SNS in the bone loss induced by removal of the mechanical stimuli, although protective effects of β -blockers on this bone loss were reported in a model of hind limb unloading. This model exhibits however several intrinsic physiological changes including alterations in the SNS activity and in neuromuscular function, rendering the interpretation of these results difficult. These findings do not rule out however that other neuronal pathways, that do not involve β -adrenergic signalling, contribute to bone adaptation to its mechanical environment, since bone is rich in sensory innervation that also affects bone metabolism⁸⁷.

While some discrepancies exist regarding the effects of β -blockers on bone mass *in vitro* and *in vivo*, there is more consistency about the anabolic effects of β -blockers on bone in animal models subjected to OVX and in cross-sectional studies of postmenopausal women, which suggests that the influence of the SNS on the regulation of bone mass is enhanced in the presence of hormonal changes such as the absence of estrogen. Similarly, the protective effects of β -blockers on the bone loss induced by unloading might be related to changes in serum leptin levels. Those findings underpin the hypothesis that the SNS acts more as a modulator of the hormonal effects on bone rather than being a direct effector. The protective effects of β -blockers on bone mass under estrogen deprivation do not however combine with physical activity, at least at trabecular bone sites, suggesting that extreme care should be given to the treatment of osteoporotic patients undergoing exercise. Further studies are needed to identify whether direct mechanical loading applied to the bone, or exercise which involves several other physiological changes including variations in SNS activity due to alterations in energy expenditure, is synergising with the SNS blockade during estrogen deprivation. For those studies suitable animal models, such as the tibia external loading model which allows measurements of the anabolic stimuli in both trabecular and cortical compartments³⁹, and use of transgenic mice with deletion of the $\beta 2$ -adrenergic receptor are now available tools. The demonstration of the importance of the systemic interaction of the SNS with osteoregulatory hormones, such as estrogen, leptin and PTH⁷², implies that their levels in plasma should be carefully monitored in animal experiments in the future. Studies using different types of β -blockers will be necessary as well to better understand the action of the three β -adrenoreceptors on bone resorption and formation. There is also a need for new prospective clinical studies on postmenopausal women to better monitor the interactions between the type of β -blocker and the other parameters which affect bone metabolism including the diet and physical activity. Studies involving the administration of various β -blockers together with the use of vibrating platforms at regimes known to affect bone mass could be considered. Finally, investigating the physiological role of the SNS in the skeleton requires new experimental and clinical approaches. Although innervation has been shown to affect fracture repair⁸⁸⁻⁹⁰, very little is known for example on the contribution of the SNS and the possible

beneficial effects of β -blockers on osteoporotic fractures healing.

In conclusion, while the SNS is not the master controller of bone metabolism, there is increasing confirmation that it is part of a complex system which significantly contributes to its regulation. There is however still much to learn about the complicated relationships between the SNS, the hormones that regulate bone mass, and mechanical loading of bone. Despite the evidence for peripheral and central neuronal regulatory components of the bone remodelling process and the multiple clinical associations between bone and nerves, the role of the nervous system in the physiology and pathology of musculoskeletal disorders has been mostly ignored. The discovery that the SNS plays a significant role in the control of bone mass may bring it into the spotlight.

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References

1. Frost H. Vital biomechanics: proposed general concepts for skeletal adaptations to mechanical usage. *Calcif Tissue Int* 1988;42:145-56.
2. Lanyon LE. Control of bone architecture by functional load bearing. *J Bone Miner Res* 1992;7:S369-75.
3. Kannus P, Sievanen H, Vuori L. Physical loading, exercise and bone. *Bone* 1996;18:S1-3.
4. Ehrlich PJ, Lanyon LE. Mechanical strain and bone cell function: a review. *Osteoporos Int* 2002;13:688-700.
5. Serre CM, Farlay D, Delmas PD, Chenu C. Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. *Bone* 1999;25:623-9.
6. Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience* 2002;113:155-66.
7. Sherman MS. The nerves of bones. *J Bone Jt Surg* 1963;45:522-8.
8. Hert J, Sklenska A, Liskova M. Effect of intermittent stress on the rabbit tibia after resection of the peripheral nerves. *Folia Morphol* 1971;XIX:378-87.
9. Cheng MZ, Zaman G, Rawlinson SCF, Pitsillides AA, Suswillo RFL, Lanyon LE. Enhancement by sex hormones of the osteoregulatory effects of mechanical loading and prostaglandins in explants of rat ulnae. *J Bone Miner Res* 1997;12:1424-30.
10. Pitsillides AA, Rawlinson SCF, Suswillo RFL, Bourrin S, Zaman G, Lanyon LE. Mechanical strain-induced NO production by bone cells: a possible role in adaptive bone remodeling? *FASEB J* 1995;9:1609-14.
11. Wada S, Kojo T, Wang Y, Ando H, Nakanishi E, Zhang M, Fukuyama H, Uchida Y. Effect of loading on the development of nerve fibers around oral implants in the dog mandible. *Clin Oral Implants Res* 2001;12:219-24.
12. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Vaisse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature* 2005;434:514-20.
13. Lerner UH, Lundberg P. Kinins and neuro-osteogenic factors. In: Bilezikian JP, Raisz LG, and Rodan GA, (editors) *Principles of Bone Biology* 2nd ed. San Diego: Academic Press; 2002. p. 773-99.
14. Chenu C. Glutamatergic innervation in bone. *Microsc Res Tech* 2002;58:70-6.
15. Chenu C. Role of innervation in the control of bone remodeling. *J Musculoskelet Neuronal Interact* 2004;4:132-4.
16. Chenu C, Marenzana M. Sympathetic nervous system and bone remodeling. *Joint Bone Spine* 2005;72:481-3.
17. Spencer GJ, Hitchcock IS, Genever PG. Emerging neuroskeletal signalling pathways: a review. *FEBS Lett* 2004;559:6-12.
18. Patel MS, Elefteriou F. The new field of neuroskeletal biology. *Calcif Tissue Int* 2007;80:337-47.
19. Sato S, Hanada R, Kimura A, Abe T, Matsumoto T, Iwasaki M, Inose H, Ida T, Mieda M, Takeuchi Y, Fukumoto S, Fujita T, Kato S, Kangawa K, Kojima M, Shinomiya K, Takeda S. *Nat Med* 2007;13:1234-40.
20. Denes A, Boldogkoi Z, Uhreczky G, Hornyak A, Rusvai M, Palkovits M, Kovacs KJ. Central autonomic control of the bone marrow: multisynaptic tract tracing by recombinant pseudorabies virus. *Neuroscience* 2005;134:947-63.
21. Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system (1987-2007). *Brain Behav Immun* 2007;21:736-45.
22. Dinunno FA, Tanaka H, Stauffer BL, Seals DR. Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented alpha-adrenergic vasoconstriction. *J Physiol* 2001;536:977-83.
23. Wesbroek I, Van der Plas A, De Rooij KE, Klein-Nulend J, Nijweide PJ. Expression of serotonin receptors in bone. *J Biol Chem* 2001;276:28961-8.
24. Bliziotis M, Eshleman A, Burt-Pichat B, Zhang XW, Hashimoto J, Wiren K, Chenu C. Serotonin transporter and receptor expression and function in osteocytic MLO-Y4 cells. *Bone* 2006;39:1313-21.
25. Chenu C, Serre CM, Raynal C, Burt-Pichat B, Delmas PD. Glutamate receptors are expressed by bone cells and are involved in bone resorption. *Bone* 1998;22: 295-9.
26. Szczesniak AM, Gilbert RW, Mukhida M, Anderson GI. Mechanical loading modulates glutamate receptor subunit expression in bone. *Bone* 2005;37:63-73.
27. Miura S, Kawanaka K, Kai Y, Tamura M, Goto M, Schiuchi T, Minokoshi Y, Ezaki O. An increase in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha)

- mRNA in response to exercise is mediated by beta-adrenergic receptor activation. *Endocrinology* 2007;148:2441-3448.
28. Fu Q, Levine BD, Pawelczyk JA, Ertl AC, Diedrich A, Cox JF, Zuckerman JH, Ray CA, Smith ML, Iwase S, Saito M, Sugiyama Y, Mano T, Zhang R, Iwasaki K, Lane LD, Buckey JC Jr, Cooke WH, Robertson RM, Baisch FJ, Blomqvist CG, Eckberg DL, Robertson D, Biaggioni I. Cardiovascular and sympathetic neural responses to handgrip and cold pressor stimuli in humans before, during and after spaceflight. *J Physiol* 2002;544:653-64.
29. Dodd SL, Powers SK, Vrabas IS, Criswell D, Stetson S, Hussain R. Effects of clenbuterol on contractile and biochemical properties of skeletal muscle. *Med Sci* 1996;28:669-76.
30. Clarkson PM, Thompson HS. Drugs and sport. Research findings and limitations. *Sports Med* 1997;24:366-84.
31. Weltman A, Pritzlaff CJ, Wideman L, Weltman L, Weltman JY, Blumer JL, Abbott RD, Hartman ML, Veldhuis JD. Exercise-dependent growth hormone release is linked to markers of heightened central adrenergic outflow. *J Appl Physiol* 2000;89:629-35.
32. Levasseur R, Sabatier JP, Potrel-Burgot C, Lecoq B, Creveuil C, Marcelli C. Sympathetic nervous system as a transmitter of mechanical loading in bone. *Joint Bone Spine* 2003;70:515-9.
33. Kondo H, Nifuji A, Takeda S, Ezura Y, Rittling SR, Denhardt DT et al. Unloading induces osteoblastic cell suppression and osteoclastic cell activation to lead to bone loss via sympathetic nervous system. *J Biol Chem* 2005;280:30192.
34. Marenzana M, De Souza R, Chenu C. Blockade of β -adrenergic signalling does not influence the bone mechano-adaptive response in mice. *Bone* 2007;41:206-15.
35. De Souza RL, Pitsillides AA, Lanyon LE, Skerry TM, Chenu C. Sympathetic nervous system does not mediate the load-induced cortical new bone formation. *J Bone Miner Res* 2005;20:2159-68.
36. Zhang P, Tanaka SM, Jiang H, Su M, Yokota H. Diaphyseal bone formation in murine tibiae in response to knee loading. *J Appl Physiol* 2006;100:1452-59.
37. Takeuchi T, Tsuboi T, Arai M, Togari A. Adrenergic stimulation of osteoclastogenesis mediated by expression of osteoclast differentiation factor in MC3T3-E1 osteoblast-like cells. *Biochem Pharmacol* 2001;61:579-86.
38. Rubin CT, Lanyon LE. Regulation of bone formation by applied dynamic loads. *J Bone Joint Surg* 1984;66A:397-402.
39. De Souza RL, Matsuura M, Eckstein F, Rawlinson SC, Lanyon LE, Pitsillides AA. Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element. *Bone* 2005;37:810-8.
40. Hamrick MW, Pennington C, Newton D, Xie D, Isaacs C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone* 2004;34:376-83.
41. Bonnet N, Benhamou CL, Brunet-Imbault B, Arlettaz A, Horcajada MN, Richard O, Vico L, Collomp K, Courteix D. Severe bone alterations under β 2 agonist treatments: bone mass, microarchitecture and strength analyses in female rats. *Bone* 2005;37:622-33.
42. Bonnet N, Brunet-Imbault B, Arlettaz A, Horcajada MN, Collomp K, Benhamou CL, Courteix D. Alteration of trabecular bone under chronic β 2 agonists treatment. *Med Sci Sports Exerc* 2005;37:1493-1501.
43. Bonnet N, Benhamou CL, Beaupied H, Laroche N, Vico L, Dolleens E, Courteix D. Doping dose of salbutamol and exercise: deleterious effect on cancellous and cortical bones in adult rats. *J Appl Physiol* 2007;102:1502-9.
44. Bourrin S, Genty C, Palle S, Gharib C, Alexandre C. Adverse effects of strenuous exercise: a densitometric and histomorphometric study in the rat. *J Appl Physiol* 1994;76:1999-2005.
45. Bonnet N, Courteix D, Benhamou CL. Leptin, central nervous system, and bone: influence of physical activity. *Joint Bone Spine* 2005;72:477-80.
46. Zeng QQ, Jee WS, Bigornia AE, King JG Jr, D'Souza SM, Li XJ, Ma YF, Wechter WJ. Time responses of cancellous and cortical bones to sciatic neurectomy in growing female rats. *Bone* 1996;19:13-21.
47. Kodama Y, Dimai HP, Wergedal J, Sheng M, Malpe R, Kutilek S, Beamer W, Donahue LR, Rosen C, Baylink DJ, Farley J. Cortical tibial bone volume in two strains of mice: effects of sciatic neurectomy and genetic regulation of bone response to mechanical loading. *Bone* 1999;25:183-90.
48. Sakata T, Sakai A, Tsurukami H, Okimoto N, Okazaki Y, Ikeda S, Norimura T, Nakamura T. Trabecular bone turnover and bone marrow cell development in tail-suspended mice. *J Bone Miner Res* 1999;14:1596-1604.
49. Amblard D, Lafage-Proust MH, Laib A, Thomas T, Rueggsegger P, Alexandre C, Vico L. Tail suspension induces bone loss in skeletally mature mice in the C57BL/6J strain but not in the C3H/HeJ strain. *J Bone Miner Res* 2003;18:561-9.
50. Hanson AM, Ferguson VL, Simske SJ, Cannon CM, Stodieck S. Comparison of tail-suspension and sciatic nerve crush on the musculoskeletal system in young-adult mice. *Biomed Sci Instrum* 2005;41:92-6.
51. McCarty R, Kvetnansky R, Kopin IJ. Plasma catecholamines in rats: daily variations in basal levels and increments in response to stress. *Physiol Behav* 1981;26:27-31.
52. Mueller PJ, Hasser EM. Enhanced sympathoinhibitory response to volume expansion in conscious hind limb-unloaded rats. *J Appl Physiol* 2003;94:1806-12.
53. Stevens HY, Meays DR, Frangos JA. Pressure gradients and transport in the murine femur upon hind limb

- suspension. *Bone* 2006;39:565-72.
54. Yirmiya R, Goshen I, Bajayo A, Kreisel T, Feldman S, Tam J, Trembovler V, Csernus V, Shohami E, Bab I. Depression induces bone loss through stimulation of the sympathetic nervous system. *Proc Natl Acad Sci USA* 2006;103:16876-81.
55. Morey-Holton ER, Halloran BP, Garetto LP, Doty SB. Animal housing influences the response of bone to space-flight in juvenile rats. *J Appl Physiol* 2000;88:1303-9.
56. Burt-Pichat B, Lafage-Proust MH, Duboeuf F, Laroche N, Serre CM, Itzstein C, Chenu C. Dramatic decrease of bone innervation density after ovariectomy. *Endocrinology* 2005;146:503-10.
57. Hott M, Deloffre P, Tsouderos Y, Marie PJ. S12911-2 reduces bone loss induced by short-term immobilization in rats. *Bone* 2003;33:115-23.
58. Thompson DD, Rodan GA. Indomethacin inhibition of tenotomy-induced bone resorption in rats. *J Bone Miner Res* 1988;3:409-14.
59. Gomez c, David V, Peet NM, Vico L, Chenu C, Malaval L, Skerry T. Absence of mechanical loading *in utero* influences bone mass and architecture but not innervation in MyoD-Myf5 deficient mice. *J Anat* 2006;210: 259-71.
60. Baker JG, Hall IP, Hill SJ. Agonist and inverse agonist actions of β -blockers at the human β 2-adrenoreceptor provide evidence for agonist-directed signaling. *Mol Pharmacol* 2003;64:1357-69.
61. Kellenberger S, Muller K, Richener H, Bilbe G. Formoterol and isoproterenol induce c-fos gene expression in osteoblast-like cells by activating β 2-adrenergic receptors. *Bone* 1998;22:471-8.
62. Pierroz DD, Baldock P, Boussein M, Ferrari SL. Low cortical bone mass in mice lacking β 1 and β 2 adrenergic receptors is associated with low bone formation and circulating IGF-1. *J Bone Miner Res* 2006;21(Suppl.1):S26.
63. Hamrick MW, Ferrari SL. Leptin and the sympathetic connection of fat to bone. *Osteoporos Int* 2007; [Epub ahead of print].
64. Apseloff G, Girten B, Walker M, Shepard DR, Krecic ME, Stern LS, Gerber N. Aminohydroxybutane bisphosphonate and clenbuterol prevent bone changes and retard muscle atrophy respectively in tail-suspended rats. *J Pharmacol Exp Ther* 1993;264:1071-8.
65. Zeman RJ, Hirschman A, Hirschman ML, Guo G, Etlinger JD. Clenbuterol, a beta 2-receptor agonist, reduces net bone loss in denervated hindlimbs. *Am J Physiol* 1991;261:E285-9.
66. Takeda S, Eleftheriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 2002;111:305-17.
67. Kitaura T, Tsunekawa N, Kraemer WJ. Inhibited longitudinal growth of bones in young male rats by clenbuterol. *Med Sci Sports Exerc* 2002;34:267-73.
68. Bonnet N, Laroche N, Beaupied H, Vico L, Dolleens E, Benhamou CL, Courteix D. Doping dose of salbutamol and exercise training: impact on the skeleton of ovariectomized rats. *J Appl Physiol* 2007;103:524-33.
69. Bonnet N, Beaupied H, Vico L, Dolleens E, Laroche N, Courteix D, Benhamou CL. Combined effects of exercise and propranolol on bone tissue in ovariectomized rats. *J Bone Miner Res* 2007;22:578-88.
70. Bonnet N, Laroche N, Vico L, Dolleens E, Benhamou CL, Courteix D. Dose effects of propranolol on cancellous and cortical bone in ovariectomized adult rats. *J Pharmacol Exp Ther* 2006;318:1118-27.
71. Minkowitz B, Boskey AL, Lane JM, Pearlman HS, Vigorita VJ. Effects of propranolol on bone metabolism in the rat. *J Orthop Res* 1991;9:869-75.
72. Pierroz DD, Boussein ML, Rizzoli R, Ferrari SL. Combined treatment with a β -blocker and intermittent PTH improves bone mass and microarchitecture in ovariectomized mice. *Bone* 2006;39:260-7.
73. Pierroz DD, Boussein M, Muzzin P, Rizzoli R, Ferrari SL. Bone loss following ovariectomy is maintained in absence of adrenergic receptor β 1 and β 2 signaling. *J Bone Miner Res* 2005;20(Suppl.1):S277.
74. Dhillon H, Glatt V, Ferrari SL, Boussein ML. β -adrenergic receptor KO mice have increased bone mass and strength but are not protected from ovariectomy-induced bone loss. *J Bone Miner Res* 2004;19(Suppl.1):1122.
75. Ito M, Nishida A, Nakamura T, Uetani M, Hayashi K. Differences of three-dimensional trabecular microstructure in osteopenic rat models caused by ovariectomy and neurectomy. *Bone* 2002;30:594-8.
76. Martin A, de Vittoris R, David V, Moraes R, Begeot M, Lafage-Proust MH, Alexandre C, Vico L, Thomas T. Leptin modulates both resorption and formation while preventing disuse-induced bone loss in tail-suspended female rats. *Endocrinology* 2005;146:3652-9.
77. Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC. β -adrenergic blockers reduce the risk of fractures partly by increasing bone mineral density: Geelong osteoporosis study. *J Bone Miner Res* 2004;19:19-24.
78. Schlienger RG, Kraenzlin ME, Jick SS, Meier CR. Use of β -blockers and risk of fractures. *JAMA* 2004;292:1326-32.
79. Rejnmark L, Vestergaard P, Kassem M, Christoffersen BR, Kolthoff N, Brixen K, Mosekilde L. Fracture risk in perimenopausal women treated with β -blockers. *Calcif Tissue Int* 2004;75:365-72.
80. Levasseur R, Dargent-Molina P, Sabatier JP, Marcelli C, Breart G. β -blocker use, bone mineral density and fracture risk in older women: results from the Epidemiologie de l'osteoporose prospective study. *J Am Geriatr Soc* 2005;53:550-2.
81. Reid IR, Gamble GD, Grey AB, Black DM, Ensrud KE, Browner WS, Bauer DC. β -blocker use, BMD and fractures in the study of osteoporotic fractures. *J Bone Miner Res* 2005;20:613-8.
82. Reid IR, Lucas J, Wattie D, Horne A, Bolland M,

- Gamble GD, Davidson JS, Grey AB. Effects of a β -blocker on bone turnover in normal postmenopausal women: a randomized controlled trial. *J Clin Endocrinol Metab* 2005;90:5212-6.
83. Turker S, Karotosun V, Gunual I. β -blockers increase bone mineral density. *Clin Orthop Relat Res* 2006;443:73-4.
84. Bonnet N, Gadois C, McCloskey E, Lemineur G, Lespessailles E, Courteix D, Benhamou CL. Protective effect of β -blockers in postmenopausal women: influence on fractures, bone density, micro and macroarchitecture. *Bone* 2007;40:1209-16.
85. De Vries F, Souverein PC, Cooper C, Leufkens HG, Van Staa TP. Use of β -blockers and the risk of hip/femur fracture in the United Kingdom and the Netherlands. *Calcif Tissue Int* 2007;80:69-75.
86. Patel MS, Elefteriou F. The new field of neuroskeletal biology. *Calcif Tissue Int* 2007;80:337-47.
87. Offley S, Guo T, Wei T, Clark JD, Vogel H, Lindsey DP, Jacobs CR, Yao W, Lane NE, Kingery WS. Capsaicin-sensitive sensory neurons contribute to the maintenance of trabecular bone integrity. *J Bone Miner Res* 2005;20:257-67.
88. Aro H. Effect of nerve injury on fracture healing. *Acta Orthop Scand* 1985;56:233-7.
89. Li J, Ahmad T, Spetea M, Ahmed M, Kreicbergs A. Bone reinnervation after fracture: a study in the rat. *J Bone Miner Res* 2001;16:1505-10.
90. Hukkanen M, Konttinen YT, Santavirta S, Paavolainen P, Gu XH, Terenghi G, Polak JM. Rapid proliferation of calcitonin gene-related peptide-immunoreactive nerves during healing of rat tibial fracture suggests neural involvement in bone growth and remodeling. *Neuroscience* 1993;54:969-79.