Adrenergic control of bone remodeling and its implications for the treatment of osteoporosis

N. Bonnet, D.D. Pierroz, S.L. Ferrari
Division of Bone Diseases, Department of Rehabilitation and Geriatrics, WHO Collaborating Center for Osteoporosis Prevention, Geneva University Hospital, Geneva, Switzerland

Abstract
Evidence that leptin regulates bone turnover in part through a central nervous system (CNS)/β-adrenergic system relay has driven attention towards the potential therapeutic benefits of β-adrenergic blockade to improve bone mass and strength. β2-adrenergic receptor-mediated signaling in osteoblasts inhibits bone formation and triggers RANKL-mediated osteoclastogenesis and bone resorption. Mouse models of adrenergic-deficiency, particularly the mouse lacking the β2-adrenergic receptor, have increased bone mass, more specifically increased trabecular bone volume. In turn, β-blockers, such as propranolol, were reported to inhibit ovariectomy-induced bone loss. In contrast, a number of experiments in mice and rats suggest that inhibition of β-adrenergic receptor-mediated signaling does not improve, and could actually be detrimental, for bone mass and microstructure. In humans, epidemiological observations suggested that users of β-blockers have higher bone mineral density (BMD) and/or a reduced risk of fractures, yet not all studies were concordant. Here we review the evidence for a role of the adrenergic system in the regulation of bone metabolism in vitro and in vivo and provide some new evidence for a dual role of β-adrenergic receptors 1 and 2 on bone turnover. Furthermore, we will examine the similarities and disparities that may exist in the effects of β-adrenergic and PTH stimulation on bone metabolism.

Keywords: Adrenergic Signaling, Bone Remodeling, Animal and Human Studies, Osteoblast, Osteoclast

Introduction
The process of bone modeling and remodeling ensures adaptation of the size, shape, microarchitecture and mineral content of the skeleton, as well as the repair of bone damage, in response to growth, aging and mechanical constraints. At the tissue level, alterations of the bone modeling activity, particularly during growth, and of the bone remodeling rate, as occurs with the menopause and aging, in turn will lead to decreased peak bone mass and osteoporosis, respectively. At the cellular level, physiological bone remodeling depends on the balance between bone resorption and bone formation, i.e., on the finely regulated and coordinated activity of osteoclasts and osteoblasts within the BMU. The development and activation of osteoblasts and osteoclasts is controlled by growth factors and cytokines produced by bone cells themselves as well as by surrounding bone marrow cells. More recently, the neuroendocrine system has been implicated in the regulation of bone remodeling.

Histological studies have shown that both bone and the periosteum receive a rich supply of sensory and sympathetic nerve fibers, whose density is the greatest around the growth plates and in the metaphysis of long bones. Nerve endings have been found to be in direct contact with bone cells, and catecholamine-containing axons have been identified near osteoblasts in vivo, suggesting a neuroendocrine regulation of bone remodeling. Recently, the role of bone innervation has regained attention with the description of a leptin-dependent central regulation of bone turnover through the β-adrenergic system. Leptin may act on a population of neurons located in the ventromedial hypothalamus, which in turn stimulate the activity of intraosseous sympathetic nerve fibers. These fibers release norepinephrine (NE), which binds to adrenergic receptors expressed on osteoblasts, thereby inhibiting their bone forming activity. Furthermore,
Adrenergic receptors are mostly known for their role in the regulation of cardiovascular, uterine and airway smooth muscle functions. However, after the histological demonstration of NE fibers on bone, pharmacological and genetic experiments have shown that both osteoblastic and osteoclastic cells possess receptors for NE. Gene expression of α1 and 2 receptors (Adra1R, Adra2R) and/or β2-adrenergic receptors (Adrb2R) has been detected by RT-PCR in human periosteum-derived osteoblastic cells (SaM-1)⁷, human osteosarcoma-derived cells (SaOS-2, HOS, MG63), mouse primary osteoblasts⁴ and human osteoclastic cells⁸. However, among all postsynaptic adrenergic receptors, Adrb2R appears to be the main, if not the only functional adrenergic receptor expressed in osteoblasts⁴,⁶,⁹,¹⁰. We further confirmed by PCR and confocal microscopy the presence of Adrb2R mRNA and protein, respectively, in rat calvarial osteoblasts, whereas neither Adrb1R nor Adrb3R expression was detected in these cells (Figure 1)¹¹. Furthermore, Adrb2R mRNA was expressed in whole extracts from mouse femur, whereas Adrb1R and Adrb3R genes were not². Adrenergic effects on osteoblasts and bone formation

Adrenergic receptors belong to the large family of seven transmembrane domain proteins that transduce signals through heterodimeric guanine-nucleotide-binding proteins (G proteins). Epinephrine was found to enhance DNA synthesis in proliferating cells and alkaline phosphatase (ALP) activity in differentiating MC3T3-E1 osteoblast-like cells by stimulating Adra1R coupled to Gi proteins¹¹. Furthermore, the extracellular signal regulated kinase (ERK) pathway was involved in cell proliferation, and the p38 mitogen-activated protein kinase (MAPK) pathway in ALP activity in response to epinephrine¹⁴. However, other studies did not confirm the expression of the Adra1R in human osteoblasts nor in vivo⁸.
Activated Adrβ2R is coupled to Gsα and adenyl cyclase (to produce cAMP). In turn, activated protein kinase A (PKA) will phosphorylate various protein targets, transcription factors, other kinases and cell surface receptors, including the Adrβ2R itself. We and others confirmed a dose-dependent increase of cAMP in response to the β-adrenergic agonist isoproterenol in primary osteoblast cultures, which can be blocked by the non-selective β-blocker propranolol as well as in Adrβ2R-deficient cells (Figure 1)6,19. In osteoblast-like cells, Adrβ2R-mediated cAMP and PKA activation leads to the expression of the immediate early gene c-fos9. The c-fos protein forms heterodimers with jun proteins, which regulate the transcription of AP-1 responsive genes such as osteocalcin, ALP and type 1 collagen as shown in rat bone cells and human osteosarcoma cells20. Hence Adrβ2R stimulation has the potential to induce osteoblast differentiation.

A bulk of in vitro observations however contrasts with these in vivo data. Hence osteoblast number and surface, bone formation rate (BFR) on trabecular surfaces, as well as cbfα1 and α1(1) collagen gene expression were decreased in wild-type mice receiving isoproterenol or a central infusion of the direct inhibitory effects of β2 adrenergic signaling on osteoblasts. The molecular mechanisms by which β-adrenergic agonists in primary osteoblast cultures, which can be blocked by the non-selective β-blocker propranolol as well as in Adrβ2R-deficient cells (Figure 1)6,19. In osteoblast-like cells, Adrβ2R-mediated cAMP and PKA activation leads to the expression of the immediate early gene c-fos9. The c-fos protein forms heterodimers with jun proteins, which regulate the transcription of AP-1 responsive genes such as osteocalcin, ALP and type 1 collagen as shown in rat bone cells and human osteosarcoma cells20. Hence Adrβ2R stimulation has the potential to induce osteoblast differentiation.

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desensitize cAMP signaling\(^3\) and provide a molecular switch towards MAPKinase signaling\(^3\). Of note, several \(\beta\)-adrenergic antagonists, including propranolol, exert partial agonist activity on the activation of MAPKinases ERKs through the recruitment of \(\beta\)-arrestins\(^3\). This recent finding may further complicate the interpretation of studies on the skeletal effects of \(\beta\)-blockers (see below).

**Table 1.** Similarities and differences between PTH and \(\beta\)-adrenergic signaling in bone.

<table>
<thead>
<tr>
<th>GPCR</th>
<th>PTH</th>
<th>(\beta)-adrenergic agonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signaling</td>
<td>cAMP-CREB &amp; Ca/IP3 MAPKs</td>
<td>cAMP-ATF4 MAPKs</td>
</tr>
<tr>
<td></td>
<td>(ERKs, mediated by (\beta)-arrestin and c-Src)</td>
<td>(ERKs, mediated by (\beta)-arrestin and c-Src)</td>
</tr>
<tr>
<td>Signaling desensitization and receptor internalization by agonist</td>
<td>++</td>
<td>(\beta)-adrenergic agonist</td>
</tr>
<tr>
<td></td>
<td>(by (\beta)-arrestin)</td>
<td>(\beta)-adrenergic agonist</td>
</tr>
<tr>
<td>Effects on osteoblast proliferation +</td>
<td>proliferation + differentiation/activation +++</td>
<td>proliferation-differentiation/activation +++</td>
</tr>
<tr>
<td></td>
<td>survival +</td>
<td>survival +</td>
</tr>
<tr>
<td>Effects on bone formation</td>
<td>+++</td>
<td>--</td>
</tr>
<tr>
<td>Cytokine production</td>
<td>RANKL, IL-6, (±PGE2)</td>
<td>RANKL, IL-6, PGE2</td>
</tr>
<tr>
<td>Effects on osteoclast</td>
<td>++++, indirect (RANKL)</td>
<td>+++, direct and indirect (RANKL)</td>
</tr>
<tr>
<td>Effects on bone resorption</td>
<td>intermittent PTH +</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>continuous PTH +</td>
<td></td>
</tr>
</tbody>
</table>

**Influence of the adrenergic system on bone mass, structure and strength: Animal studies**

The main studies describing the effects of adrenergic receptor activation on the skeleton were initiated following the discovery that leptin, a pleiotropic adipocyte-derived hormone, was a central inhibitor of bone formation\(^4\). The sympathetic nervous system (SNS) was identified as the neuronal pathway controlling osteoblast function and bone mass. The involvement of SNS in the regulation of bone mass has been demonstrated both pharmacologically and genetically.

**Genetic studies**

Based mostly on the analysis of the trabecular bone compartment, mouse models with low SNS activity, such as leptin-deficient \(ob/ob\) mice and mice deficient for dopamine \(\beta\)-hydroxylase (DbH\(^+\)), the step-limiting enzyme responsible for catecholamine synthesis, have first been reported to have a high bone mass phenotype\(^4\). However, both models suffer from multiple endocrine dysregulations, including hypercorticism, hyperinsulinemia and hypogonadism. Hence, their bone phenotypes are necessarily more complex than a mere increase in trabecular bone volume\(^3\). To delineate more precisely the role of \(\beta\)-adrenergic signaling on bone mass and microstructure, mice deficient for \(\beta\)-adrenergic receptor (s), i.e., \(A\), \(A\)-1R\(^+\), \(A\)-1R\(^-\) and \(A\)-2R\(^+\), mice, were subsequently investigated.

\(A\)-2R\(^+\) mice have a normal body weight, a normal hormonal status but an increased trabecular bone volume fraction (BV/TV) in the vertebrae and distal femur\(^6\). Furthermore, bone mass did not decrease in \(A\)-2R\(^+\) mice following OVX\(^6\), highlighting the importance of the SNS
integrity for estrogen-deficient bone loss. In contrast, \(Adr1^-R^-\) mice were briefly reported to have a normal BV/TV at 6 months of age (ibidem). Using both DXA and microcomputed tomography, we confirm here that \(Adr2^-R^-\) mice have higher vertebral and femoral BMD, trabecular BV/TV and thickness (TbTh) compared to WT littermates, as well as a greater cortical bone volume (BV) and thickness (C.Th) at the femoral mid-shaft (Figure 2). On the opposite, in \(Adr1^-R^-\) mice, we found lower vertebral BV/TV compared to WT littermates, whereas femoral BV/TV and C.Th were similar to WT (Figure 2).

Of note, single gene deletion in the \(\beta\)-adrenergic receptor system may result in phenotype(s) that represent, at least partially, an upregulation/overstimulation of the remaining \(\beta\)-adrenergic receptor subtypes, inasmuch as the loss of a specific receptor. For instance, \(Adr3^-R^-\) is upregulated in the fat tissue of \(Adr3^-R^-\) mice, which may contribute to a limited increase of adiposity in these mice. The analysis of double-deficient \(Adr1^-2^-R^-\) mice is therefore of interest. These mice have a slightly decreased body weight (-16%), and a significantly lower BMD at total body (-15%), spine (-21%) and femur (-26%) compared to WT. Their femur cross-sectional area (CSA), bone area and C.Th were all significantly decreased vs. WT (-25% to -33%). Vertebral TbN decreased by 24% with ISO, with a consequent decrease of trabecular connectivity (-45%) and

Hence, the "high bone mass" phenotype observed in adult \(Adr2^-R^-\) mice results from both a preservation of trabecular number (consistent with a lower bone resorption) and thickening of the trabeculae and cortex. The latter is consistent with an increased bone formation in these mice, but appears to require functional \(\beta1\)-adrenergic receptors. Taken together with the histological analysis presented above (see above ‘Adrenergic effects on osteoblasts and bone formation’), these observations suggest that \(\beta1\)- and \(\beta2\)-adrenergic receptors-mediated signaling acts like the yin and yang for the regulation of bone remodeling. Several questions however remain to be elucidated, including the precise role of adrenergic nerve fibers projecting into the periosteum and the influence of the SNS on bone mass growth (structure modeling) in addition to maintenance (remodeling).

**Pharmacological studies**

**Adrenergic agonists**

Studies on the effects of adrenergic agonists on bone have also led to controversial, sometimes opposite, results. On one side, isoproterenol (ISO), a \(\beta\)-agonist, markedly decreased trabecular BV/TV in mouse vertebrae (-34% versus placebo) due to a lower BFR and osteoblast number. We confirmed that ISO administered to growing mice for 8 weeks reduced BMD gain at total body (-33.5%), and spine (-64%), and modestly decreased femur lengthening (-5%) and vertex TbN decreased by 24% with ISO, with a consequent decrease of trabecular connectivity (-45%) and
increase in trabecular separation (+127%). Similar results were observed at the distal femur. At the midshaft femur, C:\Th and bone area were also both significantly decreased by ISO55. Of note, isoproterenol increased the percentage of lean mass and markedly decreased the percentage of fat (-77%), without major alterations in body weight gain compared to vehicle-treated mice. These important changes in body composition were accompanied by a decrease of leptin levels. Similar results were reported using a number of β-adrenergic agonists, namely clenbuterol and salbutamol30,33,48. Moreover, the biomechanical properties of rat bones treated with clenbuterol were significantly reduced, as shown by the loss of ultimate force (-21% versus placebo) and total energy (-22% versus placebo) using vertebral body compression and femoral three-point bending tests, despite the fact that clenbuterol had anabolic effects on muscle15.

On another side, in a preliminary study Pataki et al. showed a beneficial effect of salbutamol in OVX rats8. Their hypothesis was based on the theory of the mechanostat of Frost, where an increase in the mass or force of the muscle is accompanied by an anabolic effect on bone tissue30. Clenbuterol also accelerated the reduction of mineralization caused by lower limb denervation in young rats9. Formoterol and salbutamol both reduced the loss of dry weight and BMD of the trabecular fraction of rat distal femora in ovariectomized rats49,52. Of note, in 1980 the countries of Eastern Europe had proposed clenbuterol as a treatment against the muscular and osseous atrophy observed in certain diseases or during a long period of microgravity or immobility51,53. These studies indicate that, under certain conditions, β2-specific adrenergic agonists have the potential to exert positive effects on the skeleton, mainly through the preservation and/or increase of muscle mass (doping effect). Alternatively, we may hypothesize that non-specific β-adrenergic stimuli that would preferentially activate β1-adrenergic receptors could exert systemic (indirect) positive effects on the skeleton (see above).

Adrenergic antagonists

Several studies have investigated the implication of the SNS on bone by using β-adrenergic antagonists, particularly propranolol, a non-specific β-blocker. The beneficial effect of propranolol on bone was first demonstrated by Minkowitz et al. in a fractured rat model18. They showed that 9 weeks of low-dose propranolol treatment (0.1 mg/kg/day) increased bone formation parameters both at the periosteum and endosteum compartments18. They concluded that propranolol increased bone formation and consolidation in fracture cases. Moreover, in non-fractured animals, they showed by the torsion biomechanical test that propranolol improved the ultimate force by 33%. Others observed the same preventive effect of propranolol on BMD and trabecular bone microarchitecture in suspended or immobilized rats54,55. Takeda et al. demonstrated that propranolol could prevent the loss of vertebral trabecular bone induced by central leptin infusion and OVX in young/growing mice1. In contrast, propranolol increased trabecular thickness in the tibial metaphysis, but had no effect on BV/TV at the tibia and the vertebrae and no effect on the cortical compartment in intact mice56,57. Thus, several authors suggested that β-blockers could have site-specific effects, by postulating a differential innervation between loaded and unloaded bones50,58,59.

In adult mice, we reported that propranolol only partially rescued the bone loss following OVX, by preventing BMD decrease at total body, but not specifically at spine nor femur70. In the lumbar vertebral body, propranolol did not significantly improve microarchitectural parameters, such as BV/TV, whereas at the femoral midshaft, propranolol increased CSA and medullary area. Thus, propranolol appeared to have limited effects on OVX-induced changes of bone microarchitecture in the trabecular and cortical bone compartments by influencing both bone forming and bone resorbing processes (see above ‘Adrenergic effects on osteoblasts and bone formation’). However, when propranolol was combined to intermittent PTH, these drugs exerted synergistic effects on trabecular BV/TV and number as well as on bone formation indices (BFR, mineralizing perimeter), indicating that by uncoupling bone formation from bone resorption, propranolol has the potential to favor a positive bone mineral balance induced by PTH.

An important caveat in interpreting results from these studies resides in the variable degree of inhibition of β1- and β2-adrenergic receptor-mediated signaling obtained at various concentrations of antagonists. To approach this question, we used increasing doses of propranolol (0.1, 5 or 20 mg/kg/day, five days a week during 10 weeks) in adult rats. By hemodynamic monitoring, we demonstrated as Yaoita et al. a dose-dependent effect of propranolol in these rats60. Yet, in intact animals, we observed no significant difference on bone mineral density, microarchitecture and biomechanical properties of bone at any propranolol dose compared to placebo61. We then tested the efficiency of these various doses of β-blockers to prevent OVX-induced bone loss in rats. Trabecular BV/TV was decreased 30 to 50% in long bone metaphysis and vertebral bodies in the OVX compared to sham rats. Likewise, TbN and TbTh were decreased while osteoclast number was increased in OVX animals. In contrast, the bone mass of OVX rats treated with the lowest dose (0.1 mg/kg/day) of propranolol was not significantly different from sham-operated animals. Moreover, low-dose propranolol prevented the increase in osteoclast number in OVX rats and improved their bone formation parameters (mainly MAR) compared to rats treated with either placebo or higher dose (5 mg/kg/day) of propranolol59, consistent with the uncoupling effects of β2-adrenergic inhibition (see above ‘Adrenergic effects on osteoblasts and bone formation’). In the OVX rat model, Zhang et al. also demonstrated that propranolol treatment moderated the decrease of BMD and trabecular area, increased TbN, lowered trabecular separation to some extent as well as significantly depressing the urinary deoxyypyridinoline levels compared to placebo52,62.
In an attempt to reconcile results from pharmacological and genetic studies, we will therefore suggest that, similar to the situation in \( \text{Adr}^2 \text{R}^- \) mice, partial blockade of the adrenergic system by low doses of antagonists may be beneficial to the skeleton, mostly by limiting bone resorption and the remodeling of the cancellous bone compartment, whereas non-selective inhibition of \( \text{Adr}^1 \text{R}^- \)-mediated signaling, as obtained with high doses of propranolol and in double-deficient \( \text{Adr}^1 \text{R}^- \text{Adr}^2 \text{R}^- \) mice, will also affect bone formation and fail therefore to maintain a positive bone mineral balance.

### Effects of adrenergic system on bone in humans

There are several clinical situations in which overstimulation of the adrenergic system has been associated with a low bone mass and/or increased bone fragility: 1) users of \( \beta \)-agonists as bronchodilators (for asthma for instance) have 2-fold increased risk of hip/femur fracture. This risk remains significantly higher after adjustment for disease severity, but may be confounded by concomitant use of glucocorticoids; 2) in reflex sympathetic dystrophy, characterized by a hyper-

### Table 2. Odds ratio (OR) for prevalent fractures and BMD association in \( \beta \)-blocker users in observational, prospective and meta-analysis.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Case/control or cohort size</th>
<th>Subjects n= number of beta blocker</th>
<th>BMD associate with beta blocker</th>
<th>OR for any fracture</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observational</td>
<td>163/978</td>
<td>N=38, perimenopausal women</td>
<td>No association</td>
<td>3.3 (95% CI, 1.1-9.4) adjusted for medication, weight, activity</td>
<td>Rejnmark et al.</td>
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<tr>
<td>Observational</td>
<td>569/775</td>
<td>N=569, postmenopausal women</td>
<td>Higher BMD (total hip, UD forearm) S after adjustments for age, weight and thiazide</td>
<td>0.68 (95% CI, 0.49-0.96) adjusted for age, weight, height</td>
<td>Pasco et al.</td>
</tr>
<tr>
<td>Observational</td>
<td>18441/72778</td>
<td>N=640, women aged 30 to 79</td>
<td>No measurement</td>
<td>0.85 (95% CI, 0.77-0.93) adjusted for medication, BMI</td>
<td>Schlienger et al.</td>
</tr>
<tr>
<td>Observational</td>
<td>12160/48041</td>
<td>N=292, men aged 30 to 79</td>
<td>No measurement</td>
<td>0.66 (95% CI, 0.58-0.75) adjusted for medication, BMI</td>
<td>Schlienger et al.</td>
</tr>
<tr>
<td>Observational</td>
<td>8412</td>
<td>N=883, postmenopausal women</td>
<td>Higher BMD (total hip, os calcis) NS after adjustments for weight, thiazide, statin, estrogen use</td>
<td>0.92 (95% CI, 0.81, 1.05) unadjusted</td>
<td>Reid et al.</td>
</tr>
<tr>
<td>Prospective</td>
<td>7598</td>
<td>N=283, mean age 80.5±3.8 years</td>
<td>Higher BMD (femoral neck, ward triangle) NS after adjustments for weight, thiazide, statin, estrogen use</td>
<td>1.2 (95% CI, 0.9-1.5) adjusted for medication, weight</td>
<td>Levasseur et al.</td>
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<tr>
<td>Prospective</td>
<td>100</td>
<td>N=50, aged men &amp; women</td>
<td>Higher BMD (total hip, spine)</td>
<td>No measurement</td>
<td>Turker et al.</td>
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<tr>
<td>Observational</td>
<td>124655/373962</td>
<td>N= 35 838, aged men &amp; women</td>
<td>No measurement</td>
<td>0.91 (95% CI, 0.88-0.93) adjusted for medication, weight</td>
<td>Rejnmark et al.</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td></td>
<td>No measurement</td>
<td>0.86 (95% CI, 0.70-0.98) adjusted for medication, weight</td>
<td>Wiens et al.</td>
</tr>
<tr>
<td>Observational</td>
<td>156/944</td>
<td>N=158, women aged 41 to 96</td>
<td>Higher BMD (femoral neck, spine) S after adjustments for weight, age, statin and thiazide</td>
<td>0.58 (95% CI, 0.36-0.94) adjusted for medication, weight</td>
<td>Bonnet et al.</td>
</tr>
<tr>
<td>Observational</td>
<td>22247/22247</td>
<td>N=2013 men &amp; women UK study short use of beta blocker</td>
<td>No measurement</td>
<td>0.82 (95% CI, 0.74-0.91) adjusted for medication, weight</td>
<td>De Vries et al.</td>
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<td>Observational</td>
<td>6763/26341</td>
<td>N=4447 men &amp; women Dutch study short use of beta blocker</td>
<td>No measurement</td>
<td>0.87 (95% CI, 0.80-0.95) adjusted for medication, weight</td>
<td>De Vries et al.</td>
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<td>Observational</td>
<td>22247/22247</td>
<td>N=1446 men &amp; women UK study long use of beta blocker</td>
<td>No measurement</td>
<td>1.06 (95% CI, 0.94-1.20) adjusted for medication, weight</td>
<td>De Vries et al.</td>
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<tr>
<td>Observational</td>
<td>6763/26341</td>
<td>N=2116 men &amp; women Dutch study long use of beta blocker</td>
<td>No measurement</td>
<td>0.97 (95% CI, 0.87-1.09) adjusted for medication, weight</td>
<td>De Vries et al.</td>
</tr>
</tbody>
</table>

Studies (S=significant, NS=non-significant).
activity of the adrenergic pathway, localized osteopenia is induced by an increased bone resorption\textsuperscript{64}; 3) athletes doped with \textbeta\textsubscript{2}-agonists for their anabolic effect on muscle and catabolic effect on fat mass show bone loss and may be at increased risk of fatigue fractures. Hence, Collomp et al. reported a 4\% decrease of the bone mineral content at the lumbar spine in both sedentary and athletic men receiving 12 mg per day of oral salbutamol during 3 weeks\textsuperscript{65}.

On the opposite, in a population of 1,344 postmenopausal women with a mean age of 70, Pasco et al. found that \textbeta-blockers were associated with a reduced risk of any fracture (OR 0.68 [95\% CI, 0.49-0.96])\textsuperscript{66}. BMD at the total hip and ultradistal forearm was positively correlated with the use of \textbeta-blockers in the global population and in the fracture group after adjusting for age and weight, but not in the subgroup of women without fracture (Table 2). Schlienger et al. also suggested a protective effect of \textbeta-blockers against osteoporotic fractures\textsuperscript{67}. Their work was performed on the UK General Fracture Research Database, and included 30,601 cases, in which the OR of fractures was 0.77 among \textbeta-blockers users [95\% CI, 0.72-0.83]\textsuperscript{68}. In contrast, Rejmark et al. found no BMD differences between \textbeta-blocker users and non-users\textsuperscript{69}. Furthermore, they observed a higher fracture risk in subjects treated by \textbeta-blockers compared to a non-user group. However, they had a low statistical power for detecting differences in BMD due to a small number of subjects (n=38) and their population was younger than in previous studies (50 years old)\textsuperscript{70}. In a larger study, these authors recently reported that \textbeta-blockers were actually associated with a slightly reduced fracture risk (OR 0.91 [95\% CI, 0.88-0.93])\textsuperscript{70}. Reid et al. also found no association between bone parameters and \textbeta-blocker use after several adjustments, particularly for weight\textsuperscript{70}. Despite the general conclusion of this study that there was no association between fracture risk and \textbeta-blocker use, OR was 0.66 [95\% CI, 0.49-0.90] for hip fractures after adjustments for weight, age, thiazide, glucocorticoid and alcohol\textsuperscript{70}. Intriguingly, a similar significant reduction in hip and wrist fracture risk was observed in a subpopulation of patients using cardio-selective agents (\textbeta\textsubscript{1}-blocker) (relative risks 0.70 and 0.51, respectively)\textsuperscript{70}.

The relationship between bone markers and \textbeta-blockers has been analyzed in two studies. One was a randomized, placebo controlled trial comparing propranolol 160mg/day vs. placebo over 3 months, in which both osteocalcin and free deoxypyridinoline declined in the \textbeta-blocker group, but no change of alkaline phosphatase or C-terminal telopeptide of type I collagen (CTx) occurred. Consequently these results provided no firm evidence that \textbeta-blockers may alter bone turnover in humans\textsuperscript{71}. In the second observational study, Pasco et al. demonstrated that CTx is lower in \textbeta-blocker users versus non-users and that after 2 years of follow-up CTx was predictive of the adjusted bone loss\textsuperscript{72}. We analyzed a cohort of 944 women, including 158 women who were taking \textbeta-blockers and 341 age- and sex-matched controls. BMD at L1-L4 and femoral neck as well as calcaneus H mean (microarchitecture index) remained significantly higher in the \textbeta-blocker group compared to control after adjustments for age, weight, thiazide and statins, although adjustment for weight attenuated the difference in bone parameters between groups\textsuperscript{72}. Moreover, \textbeta-blockers were associated with BMD, cortical width and H mean parameter in both women with and without fracture\textsuperscript{73}. Consistent with previous studies, the OR for fracture was 0.64 [95\% CI, 0.45-0.83], excluding the subject treated with other medications, and the OR was 0.58 [95\% CI, 0.36-0.94] after adjustments for weight, age, HRT, thiazides, statins, corticosteroids as well as anti-osteoporotic treatments\textsuperscript{74}. As also noted by Reid et al., a substantial proportion (71\%) of our population was using cardio-selective \textbeta\textsubscript{1}-blockers\textsuperscript{70,72}.

Two meta-analyses have been conducted to analyze the relationship between \textbeta-blockers and fractures and/or BMD. A meta-analysis of seven studies assessing fracture risk in patients using \textbeta-blockers concluded that their use was associated with a 28\% reduction in hip fracture risk [95\% CI 19-37\% reduction] and a 14\% reduction in the risk for any fracture [95\% CI 2-24]\textsuperscript{75}. In contrast, another meta-analysis did not find any effect of \textbeta-blockers on fracture risk\textsuperscript{74}. In the latter study, however, patients treated with \textbeta-blockers were excluded, further suggesting that in humans the protective effects of \textbeta-blockers would be mediated through the \textbeta\textsubscript{1} adrenergic pathway.

**Conclusion**

Studies on the influence of \textbeta-adrenergic signaling on bone metabolism and the resulting effects of \textbeta-adrenergic stimulation and inhibition on bone mass, microstructure and ultimately bone strength have underscored the complexity of the regulation of bone remodeling by the SNS. Further studies will be required in both animal models and humans to definitely characterize the role of \textbeta\textsubscript{1}AR, \textbeta\textsubscript{2}AR and their interaction on the regulation of bone turnover. In turn, results from these studies could prompt the development and use of selective \textbeta-adrenergic agonists and antagonists for the treatment of osteoporosis.

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**References**


44. Pierroz DD, Muzzin P, Glatt V, Bouxsein ML, Rizzoli R, Ferrari SL. Beta 1-2-adrenergic receptor KO mice have decreased total body and cortical bone mass despite increased trabecular bone number. J Bone Miner Res 2004;19(Suppl.1):1121.


