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Deletion of β-Adrenergic Receptor 1, 2, or Both Leads to Different Bone Phenotypes and Response to Mechanical Stimulation

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ABSTRACT

As they age, mice deficient for the β2-adrenergic receptor (*Adrb2*$^{-/-}$) maintain greater trabecular bone microarchitecture, as a result of lower bone resorption and increased bone formation. The role of β1-adrenergic receptor signaling and its interaction with β2-adrenergic receptor on bone mass regulation, however, remains poorly understood. We first investigated the skeletal response to mechanical stimulation in mice deficient for β1-adrenergic receptors and/or β2-adrenergic receptors. Upon axial compression loading of the tibia, bone density, cancellous and cortical microarchitecture, as well as histomorphometric bone forming indices, were increased in both *Adrb2*$^{-/-}$ and wild-type (WT) mice, but not in *Adrb1*$^{-/-}$ nor in *Adrb1b2*$^{-/-}$ mice. Moreover, in the unstimulated femur and vertebra, bone mass and microarchitecture were increased in *Adrb2*$^{-/-}$ mice, whereas in *Adrb1*$^{-/-}$ and *Adrb1b2*$^{-/-}$ double knockout mice, femur bone mineral density (BMD), cancellous bone volume/total volume (BV/TV), cortical size, and cortical thickness were lower compared to WT. Bone histomorphometry and biochemical markers showed markedly decreased bone formation in *Adrb1b2*$^{-/-}$ mice during growth, which paralleled a significant decline in circulating insulin-like growth factor 1 (IGF-1) and IGF–binding protein 3 (IGF-BP3). Finally, administration of the β-adrenergic agonist isoproterenol increased bone resorption and receptor activator of NF-κB ligand (RANKL) and decreased bone mass and microarchitecture in WT but not in *Adrb1b2*$^{-/-}$ mice. Altogether, these results demonstrate that β1- and β2-adrenergic signaling exert opposite effects on bone, with β1 exerting a predominant anabolic stimulus in response to mechanical stimulation and during growth, whereas β2-adrenergic receptor signaling mainly regulates bone resorption during aging.

KEY WORDS: β-ADRENERGIC; MECHANICAL LOADING; PEAK BONE MASS ACQUISITION; ISOPROTERENOL

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 damages, in response to growth, aging, and mechanical constraints.[1] Alterations of the bone modeling activity, particularly during growth, and of the bone remodeling balance, as occurs with the menopause and aging, in turn will lead to decreased peak bone mass and osteoporosis, respectively.[2] The activity of osteoblasts—the bone forming cell—and osteoclasts—the bone resorbing cell—is regulated by a host of hormones and local factors,[3] among which leptin was recently shown to exert control of bone mass through the central nervous system.[4] In the arcuate nucleus of the hypothalamus, leptin inhibits bone formation and stimulates bone resorption on cancellous bone through the sympathetic nervous system (SNS).[4,5] *Ob/ob* mice, deficient in leptin and with a low sympathetic tone,[6] have increased vertebral cancellous bone mass,[4] but also reduced distal femur cancellous bone volume, cortical area, and cortical thickness.[7] Reid[8] has raised the possibility that leptin could have dual actions on different skeletal compartments due to

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diverse environment; ie, direct anabolic effects on cortical bone and indirect catabolic ones on cancellous bone mediated by the sympathetic system.

Consistent with these observations, several investigators have reported the expression of β2-adrenergic receptors, and less consistently β1-adrenergic receptors, in human periosteal osteoblasts, osteosarcoma-derived osteoblast-like cells, rat and mouse osteoblastic cells,[5,9–11] and also in human osteoclast-like cells.[12,13] Adrenergic agonists stimulated bone resorption in neonatal mouse calvariae,[10] increased osteoclastogenesis in vitro, and promoted the osteoblastic production of interleukin 6 (IL-6) and receptor activator of NF-κB ligand (RANKL)—two osteoclast-activating cytokines.[14,15] In turn, β2-adrenergic receptor deficient (*Adrb2*$^{-/-}$) mice were characterized by increased cancellous bone volume and bone formation rate (BFR) compared to their wild-type (WT) as they age (ie, by 6 months of age) and also following estrogen-deficiency, at least when ovariectomy was performed during growth (ie, by 1 month of age).[14] Thus, β2-adrenergic signaling directly inhibits bone formation and promotes osteoclastogenesis by increasing RANKL.[16] In contrast, we reported that obese β-less mice (deletion of β1-, β2-, and β3-adrenergic receptors) are not protected against cancellous bone loss during aging and with ovariectomy, suggesting that concomitant loss of β1-adrenergic receptors and/or β3-adrenergic receptors may counteract the protective effects of β2-adrenergic receptor deficiency.[17] Furthermore, some data suggest that adrenergic agonists may exert anabolic effects on bone, as shown by the partial prevention of femur demineralization in denervated rats.[18] However, no increase in cortical bone mass or strength has been demonstrated in these conditions. The effects of β-adrenergic blockade by propranolol on bone, as investigated in several rodent models, has also led to contrasting results.[19–23]

We postulated that β1-adrenergic receptors might play a role on the regulation of bone turnover and bone mass, that opposes the influence of β2-adrenergic receptors. For this purpose we evaluated the skeletal response to loading by axial compression in *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ double knockout mice and we further characterized the mechanisms by which β1-adrenergic receptors and β2-adrenergic receptors may regulate bone mass and microarchitecture.[24] Our results indicate that β1-adrenergic receptors and β2-adrenergic receptors exert opposite effects on bone remodeling through both systemic and local factors.

Subjects and Methods

Animals and experimental procedures

Adrb1b2$^{-/-}$ mice were constructed by gene-targeting techniques as previously described, and were kindly provided by Dr BK Kobilka. They were compared to WT. *Adrb1b2*$^{-/-}$ mice were on a mixed 129 SvJ, FVB/N, C57BL/6J genetic background.[24] Mice were maintained under standard non-barrier conditions and had access to mouse chow (RM3; SDS, Surrey, UK) and water *ad libitum*.

Mechanical stimulation

We submitted the left tibia of 4-month-old *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice and their respective WT to a cyclic axial compression protocol on a specific device designed and manufactured by Dr D Pioletti, EPFL, Switzerland. Mice were anesthetized with ketamine/xylazine and their left tibia was submitted to a dynamic cyclic axial compression protocol: peak load of 12 N, full-cycle frequency (pulse + rest) = 0.1 Hz, pulse period (trapeze-shaped pulse) = 0.2 seconds, peak strain (on medial midshaft cortex) = 1500 με for a total of 40 cycles (7 minutes) 3 days/week for 2 weeks. The right unloaded tibia was the paired control. After 2 weeks of mechanical stimulation, mice were euthanized and the left and right tibiae were collected for micro–computed tomography (μCT) and histology evaluation.

Generation of Adrb1$^{-/-}$ and Adrb2$^{-/-}$ mice

We crossed *Adrb1b2*$^{-/-}$ mice and their WT, and the double-heterozygous *Adrb1b2*$^{+/-}$ mice were intercrossed for six generations before the generation of the specific *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$ mice and their respective WT.

Isoproterenol treatment

Isoproterenol (Sigma, Buchs, Switzerland) was freshly dissolved in NaCl 0.9%. We injected six-week-old male *Adrb1b2*$^{-/-}$ mice and their respective WT intraperitoneally for 8 weeks with isoproterenol (5 mg/kg twice a day) or vehicle. Body weight was measured weekly and the isoproterenol dose adjusted accordingly. For each experiment, blood was obtained by retro-orbital bleeding after a fast of 6 hours at specific time-points for biochemical determinations. All animal procedure was approved by the University of Geneva School of Medicine Ethical Committee and the State of Geneva Veterinarian Office.

Measurement of bone mineral density, morphology, and microarchitecture

We measured whole-body, femoral, and spinal bone mineral density (BMD, g/cm^2), and percentage fat mass in mice by dual-energy X-ray absorptiometry (PIXImus2; GE Lunar, Madison, WI, USA) at start and end of specific interventions. Ex vivo μCT (UCT40; Scanco Medical AG, Bassersdorf, Switzerland) was used to assess cancellous bone volume fraction and microarchitecture in the excised 5th lumbar vertebral body, distal femoral metaphysis or proximal tibial metaphysis, and cortical bone geometry at the mid-femoral and mid-tibial diaphysis as described.[25,26]

Biochemical determinations

We measured serum tartrate resistant alkaline phosphatase form 5b (TRACP5b) (IDS Ltd., Tyne & Wear, UK), osteocalcin (Biomedical Technologies Inc., Stroughton, MA, USA), parathyroid hormone (PTH) (Immunotopics, Inc., San Clemente, CA, USA), leptin (Crystal Chem Inc., Chicago, IL, USA), IGF-1 (IDS Ltd.), and IGF–binding protein 3 (IGF-BP3) (R&D Systems Europe, Ltd., Abingdon, UK) according to the manufacturers' instructions. Calcium and phosphate were measured as described.[26]

Histology

To estimate bone mineralization rate, mice received subcutaneous injections of calcein (30 mg/kg; Sigma, Buchs, Switzerland) 9

and 2 days before euthanasia. We embedded femurs or tibias in methylmethacrylate (Merck, Schaffhausen, Switzerland), and we cut 8-μm-thick transversal sections of the mid-shaft with a Polycut E microtome (Leica Corp. Microsystems AG, Glattbrugg, Switzerland), then mounted them unstained for evaluation of fluorescence. Sagittal sections 5-μm-thick were stained with modified Goldner's trichrome and histomorphometric measurements were performed on the secondary spongiosa of the distal femur, using a Leica Corp. Q image analyzer. All parameters were calculated and expressed according to standard formulas and nomenclatures.[27]

Primary osteoblastic cell cultures

We obtained primary osteoblasts from neonatal calvaria of WT and *Adrb1b2*$^{-/-}$ litters. Briefly, cells were harvested by sequential collagenase type II (3 mg/mL; Sigma) digestions of calvaria from 2- to 3-day-old mice, as described.[26] At day 14 of culture, primary osteoblastic cells were stimulated with 0.1 mM isoproterenol and 0.1 nM of bovine PTH (bPTH) 1-34, or vehicle, for 1 to 6 hours, and immediately frozen for subsequent RNA extraction.

RNA isolation and gene expression by RT-PCR

We extracted total RNA from WT and *Adrb1b2*$^{-/-}$ primary osteoblastic cells and from WT and *Adrb21*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ femurs using TriPure (Roche, Basel, Switzerland), combined with DNAse treatment (RNase-free DNase Set; Qiagen, Basel, Switzerland), and purified on RNeasy Mini-column (Qiagen). Total RNA (2 μg) was reverse-transcribed using a High-Capacity cDNA Archive Kit (Applied Biosystems, Rotkreuz, Switzerland) and diluted twofold. Quantitative RT-PCR (ABI PRISM 7000) was done as follows: 2 minutes at 50°C, 10 minutes at 95°C, and cycles of PCR consisting of 0.15 minutes at 95°C and 1 minute at 60°C for 40 cycles. Reactions were performed in 25 μL containing 5 μL of cDNA, 12.5 μL of 2× TaqMan Universal

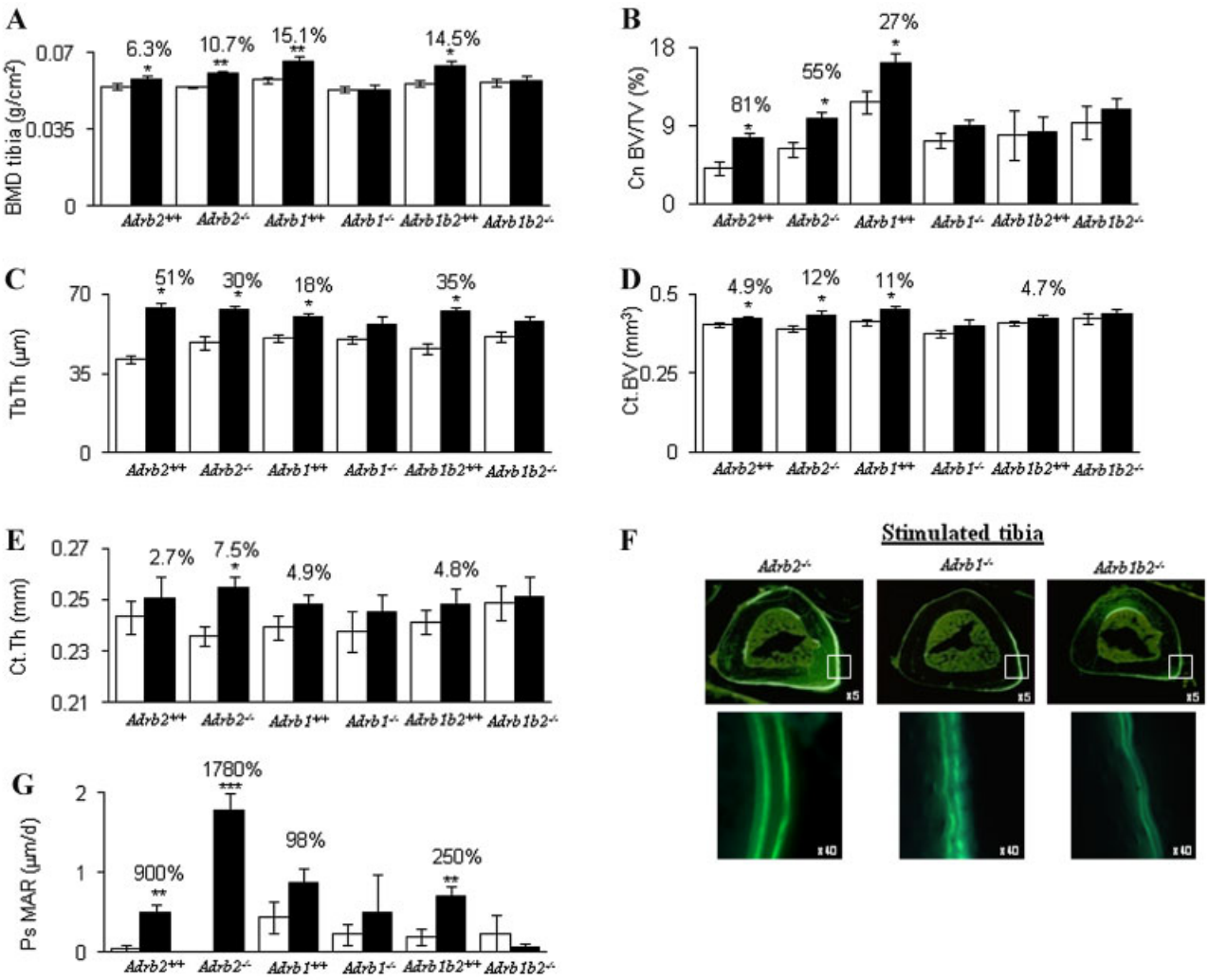

Fig. 1. Response to biomechanical stimulation in *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice. (*A*) Tibia BMD was evaluated by DXA after 2 weeks of in vivo axial compression. (*B–E*) Cancellous and cortical microarchitecture of the stimulated tibia compared to the unstimulated one, respectively at the proximal and midshaft tibia. (*F*,*G*) Bone remodeling at cortical bone surfaces in response to axial compression. In *F*, fluorescent sections of midshaft tibia showing cortical calcein labels on cortical surfaces of stimulated tibia in *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice. In *G*, mineral apposition rate (MAR) on the periosteum surface (Ps). Bars show mean (±SEM) measured after 2 weeks of axial compression in stimulated tibia (closed bars) or nonstimulated tibia (open bars), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ paired *t* test compared to nonstimulated tibia. BMD = bone mineral density; Cn.BV/TV = cancellous bone volume; Tb.Th = trabecular thickness; Ct.BV = cortical bone volume; Ct.Th = cortical thickness.

PCR Master Mix, 1.25 μL of 20× mix of predesigned primers and TaqMan MGB probes (FAM-dye labeled, Assays-on-Demand products; Applied Biosystems) and H_2O up to 25 μL. References for Assays-on-Demand are as follows: for osteoprotegerin (OPG), Mm_00435452_m1; for RANKL, Mm_00441908_m1; for IGF-1, Mm_00439559_m1; for IGF-2, Mm_00439564_m1; for adrenergic receptor β1, Mm_00431701_s1; for adrenergic receptor β3, Mm_00475698_m1; and for β2-microglobulin (B2M), Mm_00437762_m1. For adrenergic receptor β2, probe was Assay designed (Custom TaqMan Gene Expression Assay designed with File Builder 3.0 Software). mRNA level of each gene was normalized by B2M as internal standard. The relative expression of targeted mRNA was computed from the threshold cycle (Ct) values of the target and internal standard gene, according to the manufacturer's notice (User Bulletin #2, Applied Biosystems).

Statistical analysis

Data are presented as mean ± SEM. A two-factor analysis of variance was used to assess the effect of isoproterenol and adrenergic receptors β1 and β2 deficiency on skeletal morphology. As appropriate, post hoc testing was performed using Fisher's protected least squares difference (PLSD). Values of p for the differences in gene expression levels or genotype in absence of treatment were computed by t test for unpaired comparisons. Values of p for the differences between the stimulated and the nonstimulated tibia in the same animal were computed by t test for paired comparisons. Differences were considered significant at $p < 0.05$.

Results

Influence of β-adrenergic receptors on the skeletal response to mechanical loading

Adrb1$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice were subjected to 2 weeks of an in vivo axial compression of the tibia. BMD was significantly increased compared to the unstimulated tibia in the *Adrb2*$^{-/-}$ mice and their WT littermate (*Adrb2*$^{-/-}$: +10.7%, WT: +6.3%, $p < 0.05$ for both genotypes), but remained unchanged in *Adrb1*$^{-/-}$ and *Adrb1b2*$^{-/-}$ mice (Fig. 1*A*). Axial compression

Table 1. Bone Formation Indices in 4-Month-Old Male *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ Mice

Histology	Nonstimulated tibia	Stimulated tibia	p	Nonstimulated tibia	Stimulated tibia	p
	WT ($n = 5$)			*Adrb1*$^{-/-}$ ($n = 6$)		
Endocortical						
Ec MAR (μm/d)	0.33 ± 0.03	1.01 ± 0.25	<0.01	0.46 ± 0.20	0.93 ± 0.42	NS
Ec BFR/BPm (μm²/μm/d)	0.01 ± 0.001	0.05 ± 0.03	0.1	0.02 ± 0.01	0.02 ± 0.01	NS
Ec MPm/BPm (%)	0.12 ± 0.04	0.43 ± 0.16	0.1	0.26 ± 0.11	0.25 ± 0.06	NS
Periosteal						
Ps MAR (μm/d)	0.44 ± 0.19	0.87 ± 0.19	0.1	0.23 ± 0.13	0.49 ± 0.49	NS
Ps BFR/BPm (μm²/μm/d)	0.14 ± 0.07	0.21 ± 0.06	NS	0.02 ± 0.01**	0.04 ± 0.04	NS
Ps MPm/BPm (%)	0.22 ± 0.08	0.29 ± 0.09	NS	0.09 ± 0.02*	0.08 ± 0.03	NS
	WT ($n = 5$)			*Adrb2*$^{-/-}$ ($n = 6$)		
Endocortical						
Ec MAR (μm/d)	0.03 ± 0.03	1.20 ± 0.25	<0.001	0.59 ± 0.24*	1.82 ± 0.09	<0.01
Ec BFR/BPm (μm²/μm/d)	0.01 ± 0.01	0.3 ± 0.02	<0.001	0.1 ± 0.1	0.5 ± 0.2	<0.05
Ec MPm/BPm (%)	0.10 ± 0.02	0.23 ± 0.04	<0.05	0.15 ± 0.06	0.34 ± 0.07	0.05
Periosteal						
Ps MAR (μm/d)	0.05 ± 0.04	0.50 ± 0.10	<0.01	0 ± 0	1.78 ± 0.21	<0.001
Ps BFR/BPm (μm²/μm/d)	0.02 ± 0.02	0.10 ± 0.05	NS	0 ± 0	0.60 ± 0.26	<0.05
Ps MPm/BPm (%)	0.12 ± 0.08	0.18 ± 0.07	NS	0.05 ± 0.02	0.33 ± 0.14	<0.05
	WT ($n = 5$)			*Adrb1b2*$^{-/-}$ ($n = 5$)		
Endocortical						
Ec MAR (μm/d)	0.15 ± 0.07	1.10 ± 0.19	<0.001	0.27 ± 0.24	0.50 ± 0.29	NS
Ec BFR/BPm (μm²/μm/d)	0.003 ± 0.001	0.04 ± 0.01	<0.05	0.004 ± 0.004	0.01 ± 0.01	NS
Ec MPm/BPm (%)	0.11 ± 0.02	0.34 ± 0.09	<0.05	0.04 ± 0.02*	0.05 ± 0.01	NS
Periosteal						
Ps MAR (μm/d)	0.20 ± 0.1	0.70 ± 0.12	<0.01	0.23 ± 0.23	0.06 ± 0.06	NS
Ps BFR/BPm (μm²/μm/d)	0.07 ± 0.04	0.16 ± 0.04	0.1	0.02 ± 0.02	0.03 ± 0.03	NS
Ps MPm/BPm (%)	0.16 ± 0.06	0.24 ± 0.06	NS	0.04 ± 0.02*	0.05 ± 0.01	NS

All parameters were calculated and expressed according to standard formulas and nomenclatures. Values are means ± SEM. Genotype effect has been tested exclusively on the nonstimulated tibia.

WT = wild-type; Ec = endocortical; MAR = mineral apposition rate; NS = not significant; BFR/BPm = bone formation rate; MPm/BPm = mineralizing perimeter per bone perimeter; Ps = periosteal.

*$p < 0.05$ versus WT.

**$p < 0.01$ versus WT.

also increased cancellous BV/TV and cortical bone volume and thickness in *Adrb2*$^{-/-}$ (+11% and +7.5% in stimulated versus nonstimulated tibia, $p < 0.05$ all, respectively) and WT, but not in *Adrb1*$^{-/-}$ mice (*Adrb1*$^{-/-}$: +11%, p = not significant [ns]; WT: +25.7%, $p < 0.001$ compared to the unstimulated tibia) nor in *Adrb1b2*$^{-/-}$ mice (Fig. 1*B–E*).

Dynamic bone histomorphometry showed that axial compression increased bone formation in WT and *Adrb2*$^{-/-}$mice, but not in the absence of β1-adrenergic signaling (Fig. 1*F,G*; Table 1). Hence, in WT mice, axial compression increased mineral apposition rate 2.5-fold at periosteum and 6.3-fold at endocortical surfaces (Table 1). Similar observations were made in *Adrb2*$^{-/-}$ mice, whereas compression did not significantly increase any bone formation indices at endocortical or periosteal surfaces in *Adrb1b2*$^{-/-}$ and *Adrb1*$^{-/-}$ mice. Hence, these data indicate that β1-adrenergic signaling is necessary for bone anabolic responses to mechanical stimulation, whereas β2-adrenergic signaling is not.

Influence of β-adrenergic receptors on bone mass regulation

To delineate the physiological role of β-adrenergic receptors on bone remodeling, we further characterized the skeletal phenotype of young adult *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice. First, we confirmed that 4-month-old *Adrb2*$^{-/-}$ mice have a significant higher BMD, vertebral BV/TV, and Tb.Th compared to WT (Table 2). The cross-sectional area and the bone area of the femoral diaphysis were also higher in *Adrb2*$^{-/-}$ mice compared to WT, whereas BV/TV at distal femur was significantly lower. In contrast, *Adrb1*$^{-/-}$ mice exhibited a significant lower vertebral BV/TV and Tb.N as well as a lower cortical bone area and cross-sectional area at the midshaft femur compared to WT (Table 2). Of note, tibia length was similar in WT, *Adrb1*$^{-/-}$, and *Adrb2*$^{-/-}$mice. Altogether, these observations indicate that β1- and β2-adrenergic receptor–deficient mice have opposite bone phenotypes.

The influence of the double deletion of β-adrenergic receptors 1 and 2 on bone mass gain and loss with age was then evaluated in *Adrb1b2*$^{-/-}$ and WT mice. Adult *Adrb1b2*$^{-/-}$ mice had lower body weight (−14% versus WT, $p = 0.002$) and femur length (−4.6% versus WT, $p = 0.0001$ at 20 weeks), and lower whole-body, spine, and femur BMD compared to WT. Cancellous bone volume fraction (Cn.BV/TV), trabecular thickness (Tb.Th) and cortical bone size and thickness were significantly lower in *Adrb1b2*$^{-/-}$ mice compared to WT (Table 2).

However, at 4 weeks of age, BMD was not different between *Adrb1b2*$^{-/-}$ and WT mice, indicating that β-adrenergic signals play no major role on bone development in utero and/or postnatally. Then, in parallel to their stunted growth, *Adrb1b2*$^{-/-}$ mice developed significant lower whole-body and femur BMD compared to WT, which was detectable from 8 to 52 weeks of age, and significantly lower spine BMD, which persisted up to 32 weeks of age but not thereafter (Supplemental Table 1, Fig. 2*A*). As *Adrb1b2*$^{-/-}$ mice reached 1 year of age, differences in cancellous BV/TV and Tb.Th persisted at the distal femur, but were attenuated in vertebra (Table 2). Hence in absence of β1- and β2-adrenergic signaling, cancellous and cortical bone mass acquisition were impaired, whereas age-related bone loss was attenuated, at least in the axial skeleton (Fig. 2*B*, Table 2). The predominantly low bone mass phenotype in these *Adrb1b2*$^{-/-}$ double knockout mice was opposite to the high cancellous bone mass in adult *Adrb2*$^{-/-}$ mice, and further indicates an

Table 2. Body Composition and Microarchitecture in 4-Month-Old *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ Mice

	WT ($n = 10$)	*Adrb1*$^{-/-}$ ($n = 10$)	WT ($n = 12$)	*Adrb2*$^{-/-}$ ($n = 11$)	WT ($n = 8$)	*Adrb1b2*$^{-/-}$ ($n = 6$)
Body weight (g)	24.3 ± 0.5	22.5 ± 1.8	22.9 ± 0.5	22.6 ± 0.8	28.7 ± 0.9	22.6 ± 0.8***
Total body BMD (mg/cm^2)	55 ± 1	53 ± 1	53 ± 1	56 ± 1*	54 ± 1	45 ± 1***
Spine BMD (mg/cm^2)	83 ± 2	82 ± 3	76 ± 3	83 ± 3	78 ± 2	62 ± 2***
Femur diaphysis BMD (mg/cm^2)	81 ± 2	78 ± 2	77 ± 2	82 ± 2*	84 ± 3	60 ± 2***
Vertebral cancellous bone						
Cn.BV/TV (%)	20.1 ± 0.9	17.1 ± 1.1*	17.2 ± 1.3	21.8 ± 1.6*	27.6 ± 1.5	25.6 ± 0.4
Tb.N (mm^{-1})	3.33 ± 0.08	3.02 ± 0.1*	3.37 ± 0.1	3.36 ± 0.1	4.46 ± 0.17	5.10 ± 0.11*
Tb.Th (μm)	57 ± 1	54 ± 2	61 ± 2	57 ± 1*	56.4 ± 1.4	50.8 ± 0.6**
Femoral cancellous bone						
Cn.BV/TV (%)	8.5 ± 1.3	8.9 ± 1.0	8.2 ± 0.7	6.3 ± 0.6*	13.8 ± 1.4	12.1 ± 0.5
Tb.N (mm^{-1})	3.41 ± 0.1	3.49 ± 0.1	3.57 ± 0.1	3.22 ± 0.1*	4.09 ± 0.31	4.44 ± 0.9
Tb.Th (μm)	52 ± 2	51 ± 3	52 ± 2	49 ± 1	51.9 ± 0.2	46.7 ± 0.4#
Femur diaphysis cortical bone						
Cross-sectional area (mm^2)	1.44 ± 0.05	1.34 ± 0.03*	1.38 ± 0.05	1.52 ± 0.04*	1.86 ± 0.06	1.19 ± 0.0***
Bone area (mm^2)	0.84 ± 0.02	0.79 ± 0.02 ($p = 0.07$)	0.77 ± 0.02	0.85 ± 0.01*	0.93 ± 0.04	0.63 ± 0.0***
Medullary area (mm^2)	0.61 ± 0.03	0.56 ± 0.02	0.61 ± 0.04	0.67 ± 0.04	0.93 ± 0.04	0.56 ± 0.01***
Ct.Th (μm)	244 ± 5	239 ± 5	230 ± 3	241 ± 6	222.4 ± 6.6	192.3 ± 5.8*

n = number of mice; BMD = bone mineral density; Cn.BV/TV = cancellous bone volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Ct.Th = cortical thickness.

*$p < 0.05$,

**$p < 0.005$,

***$p < 0.0001$ versus WT.

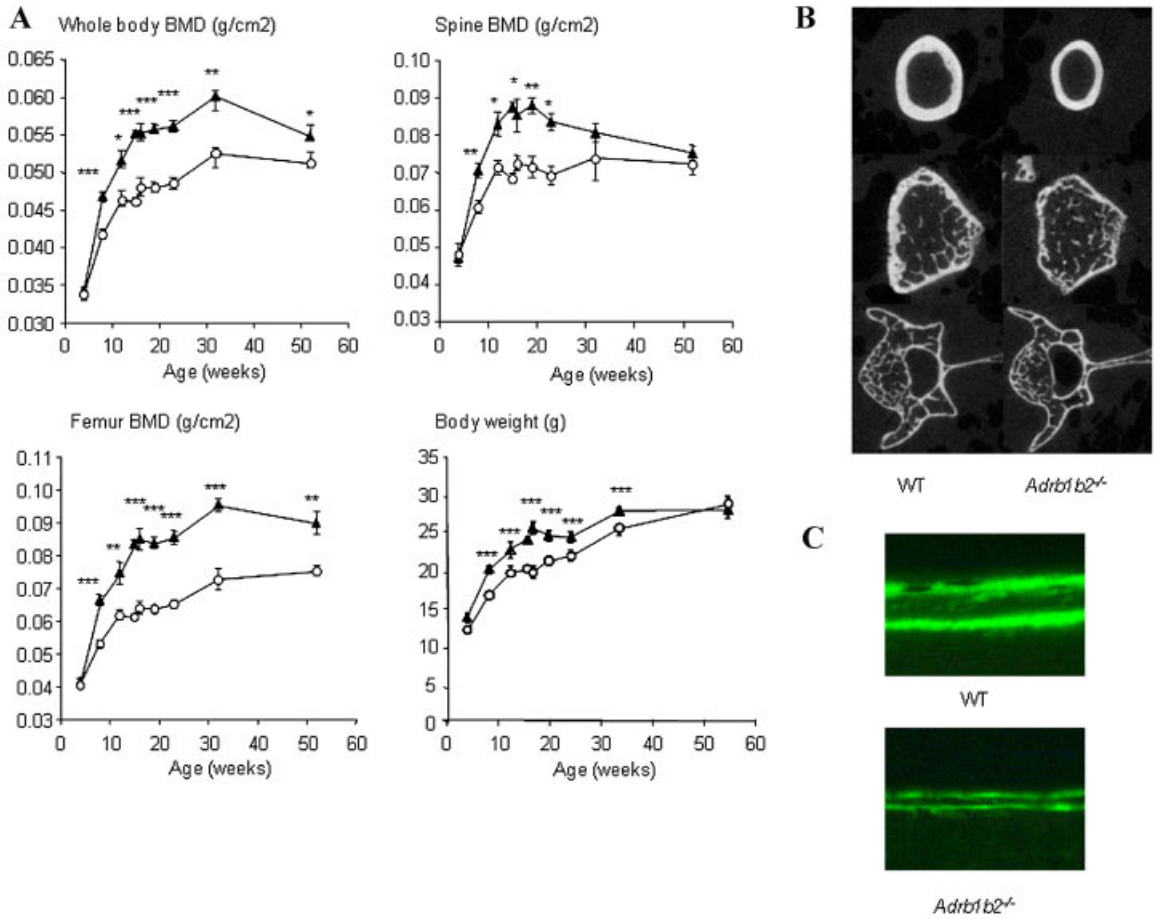

Fig. 2. Bone in *Adrb1b2*$^{-/-}$ mice. (*A*) Whole-body, spine, and femur BMD and body weight were evaluated by DXA from 4 to 52 weeks of age in WT (black triangle) and *Adrb1b2*$^{-/-}$ (empty circle) mice ($n = 6$–21/group), $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0001$ compared to WT. (*B*) 2D μCT images of the femoral diaphysis and metaphysis and the vertebra. (*C*) Decreased calcein labeling of the cortical bone (magnification ×20) in growing *Adrb1b2*$^{-/-}$ compared to WT mice.

independent role of *Adrb1* signaling on physiological bone modeling and remodeling.

Gene expression of β-adrenergic receptors

First, we confirmed by quantitative real-time RT-PCR that in WT mice, the *Adrb2* gene is more abundantly expressed in bone than *Adrb1* or *Adrb3* (data not shown). We then measured *Adrb2* gene expression in different bone compartments and found that *Adrb2* is expressed equally in the epiphysis/metaphysis and the diaphysis compartments (0.72 ± 0.12 arbitrary units and 0.78 ± 0.18 arbitrary units, respectively), and more abundantly in the bone marrow (2.13 ± 0.10 arbitrary units). Similar results were obtained for *Adrb1* gene expression, suggesting that both *Adrb1* and *Adrb2* genes could be expressed not only by bone cells but also by other bone marrow cell types. Then we evaluated whether β-adrenergic receptor subtypes would be overexpressed in the bone of *Adrb1*$^{-/-}$ and *Adrb2*$^{-/-}$ mice. *Adrb1* gene expression was increased twice in bone of *Adrb2*$^{-/-}$, whereas *Adrb2* was not overexpressed in *Adrb1*$^{-/-}$ mice. *Adrb3* gene expression was undetectable (Supplemental Table 2).

Bone turnover and biochemistry in *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice

Eight-week-old *Adrb1b2*$^{-/-}$ mice showed a marked decrease in indices of bone formation (mineral apposition rate and bone formation rate) on both cancellous and cortical periosteal surfaces (Table 3, Fig. 2*C*). Osteoblast number and surface were similar in *Adrb1b2*$^{-/-}$ and WT mice, precluding a deficit in osteoblast recruitment or proliferation. In contrast, osteoclast number and surfaces were significantly lower in the *Adrb1b2*$^{-/-}$ mice (Table 3).

Serum Pi, Ca, and PTH levels were similar in *Adrb1b2*$^{-/-}$ and WT mice (data not shown). In keeping with the histomorphometric analyses, osteocalcin was significantly lower in both young and mature *Adrb1b2*$^{-/-}$ mice compared to WT, whereas TRACP5b—an osteoclastic marker—was lower in adult *Adrb1b2*$^{-/-}$ mice compared to WT. In *Adrb1*$^{-/-}$ mice, TRACP5b was increased whereas osteocalcin was unchanged (Table 4). There was no difference in serum leptin levels between *Adrb1b2*$^{-/-}$ and WT mice. Considering the reduced body size and bone mass in *Adrb1b2*$^{-/-}$ mice and the fact that serum IGF-1 directly regulates body and skeletal growth and peak bone mass

Table 3. Quantitative Histomorphometric Indices in Cortical and Trabecular Femur in 8-Week-Old Male WT and *Adrb1b2*$^{-/-}$ Mice

Histology	WT ($n = 6$)	*Adrb1b2*$^{-/-}$ ($n = 5$)
Cancellous		
MS/BS (%)	20.47 ± 1.53	17.76 ± 1.53
MAR (μm/d)	4.25 ± 0.09	$3.42 \pm 0.07^{***}$
BFR/BS (μm^2/μm^3/d)	0.87 ± 0.06	$0.61 \pm 0.10^{*}$
ObS/BS (%)	16.71 ± 1.98	15.68 ± 2.29
ObN/BPm (/mm)	9.90 ± 0.40	10.36 ± 0.96
OcS/BS (%)	18.31 ± 1.18	$14.20 \pm 0.49^{*}$
OcN/BPm (/mm)	5.73 ± 0.53	$4.17 \pm 0.21^{*}$
Cortical		
Ps.MAR (μm/d)	2.90 ± 0.22	$1.13 \pm 0.13^{**}$
Ps.BFR/BPm (μm^2/μm/d)	2.51 ± 0.38	$0.53 \pm 0.14^{**}$

Values are mean ± SEM. Histomorphometric indices of bone remodeling were evaluated on cross-sectional sections of the mid-shaft femur and on trabecular bone of the secondary spongiosa of the distal femur as described in Subjects and Methods.

WT = wild-type; n = number of mice; MS = mineralizing surface; BS = bone surface; MAR = mineral apposition rate; BFR = bone formation rate; ObS = osteoblast surface; ObN = osteoblast number; BPm = bone perimeter; OcS = osteoclast surface; OcN = osteoclast number, Ps = periosteal.

$^{*}p < 0.05$,

$^{**}p < 0.005$,

$^{***}p < 0.0001$ for genotype by t test.

acquisition,[28–30] we measured serum IGF-1 and IGF-BP3. IGF-1 was lower in *Adrb1*$^{-/-}$ and *Adrb1b2*$^{-/-}$ mice (Fig. 3*A*), although differences in IGF-1 levels in *Adrb1b2*$^{-/-}$ mice were attenuated with age. In contrast, similar levels were measured in *Adrb2*$^{-/-}$ and WT mice. IGF-BP3 was also lower in *Adrb1b2*$^{-/-}$ mice (not determined in *Adrb1*$^{-/-}$) (Table 4).

Gene expression in *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice

To further elucidate the molecular mechanism by which adrenergic signaling regulates bone turnover, we assessed the expression of some osteoblastic genes in response to isoproterenol in *Adrb1b2*$^{-/-}$ mice. IGF-1 mRNA expression was similar in WT and *Adrb1b2*$^{-/-}$ primary osteoblastic cells at baseline (WT: 2.3 ± 0.60; *Adrb1b2*$^{-/-}$ 3.47 ± 1.31, p = ns) and increased in WT but not in *Adrb1b2*$^{-/-}$ cells after exposure to isoproterenol. In contrast, PTH significantly increased IGF-1 expression in WT and *Adrb1b2*$^{-/-}$ cells (Table 5). Furthermore, we measured IGF-2 and IGF-BP3 mRNA levels in bones. IGF-2 (WT: 1.01 ± 0.02; *Adrb1b2*$^{-/-}$: 1.04 ± 0.16 arbitrary units) and IGF-BP3 gene expression (WT: 6.8 ± 1.1; *Adrb1b2*$^{-/-}$: 6.3 ± 1.3) was similar in both genotypes. Altogether these data indicate that low bone mass in *Adrb1b2*$^{-/-}$ mice was associated with low systemic IGF-1 levels, rather than low IGF-1 or IGF-2 expression in bone.

Bone response to isoproterenol

To further evaluate the role of the β1/β2 adrenergic receptors on bone turnover, we administered isoproterenol, a nonspecific β-adrenergic receptor agonist, for 8 weeks to WT and *Adrb1b2*$^{-/-}$ mice. In WT, isoproterenol significantly decreased whole-body, spine, and femur BMD, as well as fat mass (Table 6). In these animals, isoproterenol decreased trabecular BV/TV (−14.1% and −28.3% versus vehicle, at vertebrae and distal femur, respectively), number (−14% and −26.5%, respectively), and connectivity (−19.6% and −36.7%, respectively), which was associated with an apparent increase of thickness in the remaining trabeculae. Isoproterenol also significantly inhibited cortical bone cross-sectional area (−10.4%), bone area (−13%), and cortical thickness (−8%) in WT mice. As expected, isoproterenol had no effect on bone and fat mass, trabecular and cortical architecture in *Adrb1b2*$^{-/-}$ mice (Table 6).

Isoproterenol significantly increased serum TRACP5b in WT, but not in *Adrb1b2*$^{-/-}$ mice. In contrast, isoproterenol did not modify osteocalcin level, neither in WT nor in *Adrb1b2*$^{-/-}$ mice (Fig. 3*B*), indicating that a nonspecific agonist of β1- and β2-adrenergic receptors causes bone loss through resorption.

Isoproterenol significantly decreased OPG mRNA in WT but not in *Adrb1b2*$^{-/-}$ cells and tended to increase RANKL mRNA in WT osteoblasts, but not in *Adrb1b2*$^{-/-}$ cells, leading to a significant RANKL/OPG response in WT but not in *Adrb1b2*$^{-/-}$ cells (Table 5, Fig. 3*C*). Importantly, the RANKL/OPG response to PTH was similar in WT and in *Adrb1b2*$^{-/-}$ mice, indicating that

Table 4. Serum Biochemistry in *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ Mice and Their WT

	6–9 weeks		14–16 weeks					
	WT	*Adrb1b2*$^{-/-}$	WT	*Adrb1b2*$^{-/-}$	WT ($n = 8$)	*Adrb1*$^{-/-}$ ($n = 6$)	WT ($n = 8$)	*Adrb2*$^{-/-}$ ($n = 8$)
Osteocalcin (ng/mL)	366.8 ± 13.9	$314.5 \pm 13.1^{*}$	129.5 ± 4.3	$104.0 \pm 6.6^{*}$	63.6 ± 5.4	71.0 ± 7.8	59.1 ± 4.8	63.5 ± 6.8
TRACP5b (U/L)	15.5 ± 1.3	18.5 ± 1.4	19.9 ± 1.6	$14.0 \pm 1.4^{*}$	3.0 ± 0.3	$7.2 \pm 0.7^{***}$	3.7 ± 0.2	3.6 ± 0.2
Leptin (ng/mL)	2.96 ± 0.31	2.56 ± 0.24	ND	ND	ND	ND	ND	ND
IGF-1 (ng/mL)	582.4 ± 41.0	$243.6 \pm 33.8^{***}$	692.3 ± 8.1	$458.1 \pm 31.8^{*}$	628.1 ± 154.7	$342.5 \pm 17.6^{*}$	450.3 ± 58.9	587.3 ± 99.2
IGF-BP3 (ng/mL)	716.5 ± 58.7	$208.7 \pm 14.1^{***}$	656.8 ± 4.0	$325.3 \pm 51.0^{**}$	ND	ND	ND	ND

WT = wild-type; n = 4–29 serum samples per determination; TRACP5b = tartrate resistant alkaline phosphatase form 5b; ND = not determined; IGF-1 = insulin-like growth factor 1; IGF-BP3 = IGF–binding protein 3.

$^{*}p < 0.05$,

$^{**}p < 0.005$,

$^{***}p < 0.0001$ for genotype by t test.

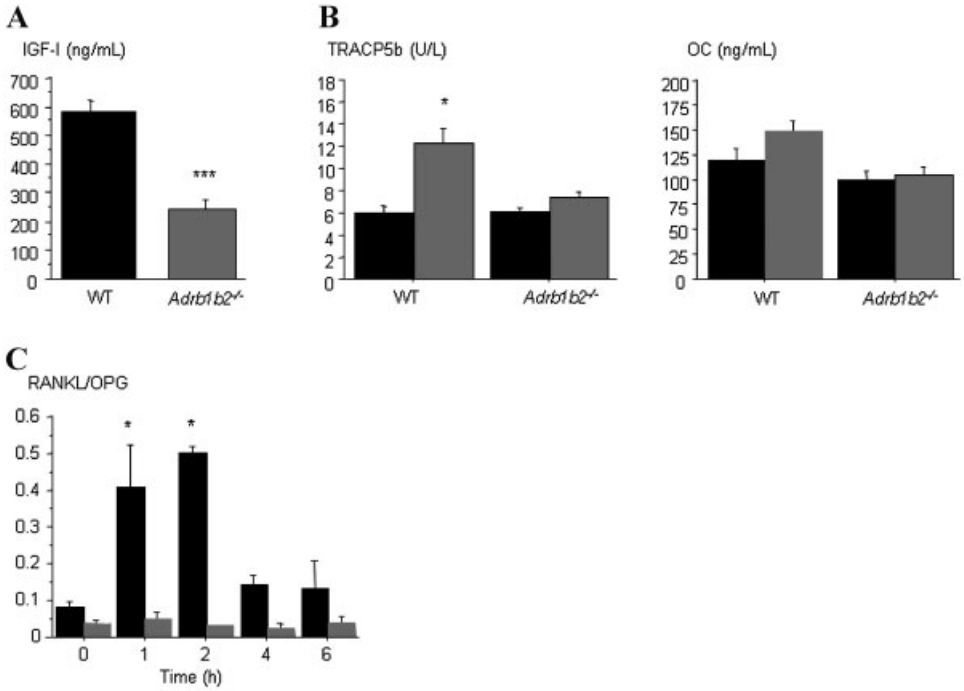

Fig. 3. (*A*) IGF-1 levels in *Adrb1b2*$^{-/-}$ and WT mice. (*B*) Serum TRACP5b and osteocalcin levels in WT and *Adrb1b2*$^{-/-}$ mice treated with vehicle (black) or isoproterenol (ISO) (gray) for 6 weeks, $^{*}p < 0.05$ versus WT. (*C*) Increased RANKL/OPG mRNA expression by ISO in primary osteoblastic WT cells (black), but not in *Adrb1b2*$^{-/-}$ (gray) mice ($n = 3$ independent cultures), $^{*}p < 0.05$ by *t* test.

the absence of RANKL stimulation in *Adrb1b2*$^{-/-}$ cells did not result from a cell autonomous defect. Hence the lack of RANKL response of *Adrb1b2*$^{-/-}$ osteoblasts to adrenergic stimuli could explain the lower osteoclast number and bone resorption, leading to preservation of vertebral trabecular bone with aging in these mice.

Discussion

By analyzing the skeletal phenotype of the *Adrb1*$^{-/-}$, *Adrb2*$^{-/-}$, and *Adrb1b2*$^{-/-}$ mice, we show that in *Adrb1*$^{-/-}$ mice, cancellous BV/TV and cross-sectional area of the mid-diaphysis were lower compared to WT littermates, and their bone-forming indices were significantly decreased, particularly at the periosteum and in response to axial compression. These observations strongly contrast with *Adrb2*$^{-/-}$ mice, which do not present a bone phenotype during growth and develop a higher trabecular bone volume by about 6 months of age.[14] Here we also confirm the high vertebral bone mass phenotype of *Adrb2*$^{-/-}$ mice and further demonstrate that, contrarily to *Adrb1*$^{-/-}$ and *Adrb1b2*$^{-/-}$ mice, the anabolic response of the peripheral skeleton to axial compression is not altered in *Adrb2*$^{-/-}$ mice. The concomitant absence of β1- and β2-adrenergic signaling leads to low bone mass and low cancellous and cortical microarchitecture. However, cancellous bone volume in the vertebrae tends to recover with aging compared to WT. The longitudinal follow-up of bone mass acquisition and maintenance indicated that β-adrenergic signaling was important for peak bone mass acquisition. Specifically, we showed that the absence of β1, 2 adrenergic receptor-mediated signaling affected mid-femoral cortical bone microarchitecture. These findings were further supported by histomorphometric analyses showing a reduced cancellous and periosteal bone formation rate in these animals, both during growth and in response to mechanical stimulation. Therefore, it appears that β1- and β2-adrenergic signaling exert opposite effects on bone. Altogether, our data indicate that β1-adrenergic signaling plays an independent and predominant role in the regulation of bone mass acquisition during growth and in response to loading, while β2-adrenergic signaling seems to regulates bone remodeling, the latter through direct effects on OPG/RANKL expression in osteoblasts.[15,31]

Because adrenergic receptors are present not only in bone cells, but also in different cells types from the bone marrow[32] and in a variety of tissues, it was important to determine whether

Table 5. Gene Expression in Osteoblastic Cells From WT and *Adrb1b2*$^{-/-}$ Mice

	WT		*Adrb1b2*$^{-/-}$	
	ISO 0.1 μM	PTH 0.1 nM	ISO 0.1 μM	PTH 0.1 nM
IGF-1	$2.61 \pm 0.55^{*}$	$5.79 \pm 1.23^{*}$	2.00 ± 0.53	$4.18 \pm 0.59^{*}$
OPG	$0.76 \pm 0.10^{*}$	$0.52 \pm 0.07^{*}$	1.23 ± 0.10	$0.26 \pm 0.04^{*}$
RANKL	4.67 ± 1.03	$16.50 \pm 5.30^{*}$	1.45 ± 0.24	$21.67 \pm 5.49^{*}$

mRNA was quantified by real-time PCR as described in Subjects and Methods in three independent cultures of calvaria derived-osteoblastic cells from each genotype stimulated by isoproterenol at 0.1 μM for 2 hours or PTH at 0.1 nM for 4 hours. Results are expressed as fold increase compared to vehicle-treated cells.

WT = wild-type; ISO = isoproterenol; PTH = parathyroid hormone; IGF-1 = insulin-like growth factor 1; OPG = osteoprotegerin; RANLK = recep-receptor activator of NF-κB ligand.

$^{*}p < 0.05$.

Table 6. Body Composition and Microarchitecture in Male WT and *Adrb1b2*$^{-/-}$ Mice Treated With Isoproterenol for 8 Weeks

	WT		*Adrb1b2*$^{-/-}$	
	Vehicle ($n = 8$)	Isoproterenol ($n = 12$)	Vehicle ($n = 6$)	Isoproterenol ($n = 7$)
Body weight (g)	28.7 ± 0.9	27.0 ± 0.6	$22.6 \pm 0.8^{\#\#\#}$	23.5 ± 1.0
Whole body BMD (mg/cm^2)	53.8 ± 0.9	$50.4 \pm 0.5^{**}$	$44.5 \pm 0.6^{\#\#\#}$	46.4 ± 0.6
Spine BMD (mg/cm^2)	78.3 ± 1.8	$70.7 \pm 1.5^{*}$	$62.1 \pm 2.2^{\#\#\#}$	65.7 ± 1.4
Femur diaphysis BMD (mg/cm^2)	83.6 ± 2.8	$76.3 \pm 1.7^{*}$	$59.7 \pm 2.0^{\#\#\#}$	62.1 ± 2.4
Fat (%)	28.6 ± 0.9	$27.0 \pm 0.6^{***}$	$22.6 \pm 0.8^{\#\#\#}$	23.4 ± 1.0
Vertebral cancellous bone				
Cn.BV/TV (%)	27.6 ± 1.5	$23.7 \pm 0.8^{*}$	25.6 ± 0.4	28.3 ± 0.7
Tb.N (mm^{-1})	4.46 ± 0.17	$3.84 \pm 0.16^{*}$	$5.10 \pm 0.11^{\#}$	5.46 ± 0.09
Tb.Th (μm)	56.4 ± 1.4	55.3 ± 1.0	$50.8 \pm 0.6^{\#\#}$	52.1 ± 0.8
Tb.Sp (μm)	235 ± 9	$277 \pm 13^{*}$	$198 \pm 6^{\#\#\#}$	182 ± 5
Connectivity density (mm^{-3})	211 ± 7	$170 \pm 8^{**}$	$246 \pm 10^{\#}$	253 ± 7
Distal femur cancellous bone				
Cn.BV/TV (%)	13.8 ± 1.4	$9.9 \pm 0.7^{*}$	12.1 ± 0.5	13.3 ± 1.0
Tb.N (mm^{-1})	4.09 ± 0.31	$2.60 \pm 0.19^{**}$	4.44 ± 0.9	4.44 ± 0.15
Tb.Th (μm)	51.9 ± 0.2	$57.1 \pm 0.1^{*}$	$46.7 \pm 0.4^{\#}$	49.9 ± 0.8
Tb.Sp (μm)	258.7 ± 28.9	$422.1 \pm 33.4^{**}$	223.8 ± 4.6	225.6 ± 7.9
Connectivity density (mm^{-3})	119 ± 17	$75 \pm 7^{*}$	122 ± 10	111 ± 12
Femur diaphysis cortical bone				
Cross sectional area (mm^2)	1.86 ± 0.06	$1.67 \pm 0.03^{*}$	$1.19 \pm 0.0^{\#\#\#}$	1.32 ± 0.04
Bone area (mm^2)	0.93 ± 0.04	$0.81 \pm 0.02^{**}$	$0.63 \pm 0.0^{\#\#\#}$	0.68 ± 0.02
Medullary area (mm^2)	0.93 ± 0.04	0.85 ± 0.02	$0.56 \pm 0.01^{\#\#\#}$	0.63 ± 0.02
Ct.Th (μm)	222.4 ± 6.6	$204.3 \pm 2.5^{*}$	$192.3 \pm 5.8^{\#}$	193.9 ± 3.3

WT = wild-type; n = number of mice; BMD = bone mineral density; Cn.BV/TV = cancellous bone volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness.

$^{*}p < 0.05$,
$^{**}p < 0.005$,
$^{***}p < 0.0001$ compared to vehicle in the same genotype.
$^{\#}p < 0.05$,
$^{\#\#}p < 0.005$,
$^{\#\#\#}p < 0.0001$ compared to WT.

the low bone mass of *Adrb1b2*$^{-/-}$ mice resulted from the alteration of local and/or systemic factors. Among the molecules regulating peak bone mass acquisition and longitudinal growth, IGF-1 plays a central role. Indeed, the growth hormone (GH)/IGF-1 axis is upregulated during the pubertal period[33,34]; serum IGF-1 levels are directly related to bone growth and peak bone mass acquisition[28–30]; IGF-1 is the most abundant growth factor synthesized and stored in bone[35–37]; and physiological levels of IGF-BP have anabolic effects on bone.[38] Accordingly, we found that serum IGF-1 and IGF-BP3 were markedly decreased in young *Adrb1b2*$^{-/-}$ mice compared with WT, whereas IGF-1 and IGF-2 mRNA levels in bone were normal, suggesting an inhibition of the somatotropic axis and/or a downregulation of liver IGF-1 production in absence of β1- and β2-adrenergic signaling. The adrenergic system is known to influence the somatotropic axis.[39–42] Because *Adrb2*$^{-/-}$ mice do not present alterations in bone mass acquisition or growth, whereas *Adrb1*$^{-/-}$ mice do, we conclude that β1-adrenergic signaling is involved in the regulation of systemic IGF-1 levels. The bone phenotype of *Adrb1b2*$^{-/-}$ mice characterized by the bone microarchitectural and histomorphometric data are reminiscent of the skeletal alterations observed in liver-specific IGF-1 gene-deficient (LID) mice, suggesting a potential alteration in the IGF-1 axis in *Adrb1b2*$^{-/-}$ mice. This hypothesis is further supported by previous pharmacological experiments showing that β1 and/or 2–mediated signaling influences GH-IGF-1 activity.[43,44] Thus, absence of adrenergic signaling through β1- and β2-adrenergic receptors leads to decreased bone formation and IGF-1during growth, lower bone mass during the entire lifetime, and reduced bone turnover. Whether normalization of serum IGF-1 levels during growth would be sufficient to fully restore the bone phenotype of the *Adrb1b2*$^{-/-}$ mice remains to be elucidated.

Although the SNS seems to be implicated in the bone adaptation to loading, the data are conflicting.[45] First, contrasting effects of SNS activation have been reported on bone loss induced by immobility; ie, by tail suspension or sciatic neurectomy.[20,23] Nevertheless, the main and recent data indicate that β-adrenergic blockade mitigates bone loss induced by disuse.[21] These effects could actually be explained by an inhibition of RANKL expression, as seen in β-adrenergic–deficient osteoblasts. On the other hand, propranolol had no effects on cortical or cancellous bone formation induced by axial compression (supraphysiological mechanical stimulation),[23] but

reduced cortical porosity and improve mechanical strength in trained ovariectomized rats (considered as a physiological mechanical stimulation).[22] The use of different doses of propranolol in these studies is critical due to the paradoxical and nonselective effects of propranolol on adrenergic receptors.[46] In our study, by selectively removing the β1- and β2-adrenergic receptors or both, we showed that β1-adrenergic signaling was the most important for cancellous and cortical responses to mechanical stimulation. Note, however, that we only found low expression levels of *Adrb1* mRNA in osteoblasts from calvaria of WT mice. It remains possible that mechanical stimulation induces the expression of *Adrb1* osteoblasts and/or osteocytes. It is also plausible that low systemic IGF-1 levels in absence of *Adrb1* negatively condition the osteoblastic response to mechanical loads. It has also been previously shown that in vivo mechanical stimulation increases IGF-1 and IGF-BP-2 mRNA in osteocytes.[47–49] In contrast, deletion of the β2-adrenergic receptor seems to play a minimal role in the mechanoadaptive response, in agreement with Marenzana and colleagues.[23] Moreover, as shown in *Adrb1b2*$^{-/-}$ mice, the absence of the β2-adrenergic receptor did not rescue the low anabolic response to loading caused by *Adrb1* deficiency, further indicating that β1- and β2-adrenergic signaling regulate bone modeling and remodeling, respectively, by independent and noncompensatory pathways.

These data may have clinical implications, since nonselective β blockers, and more commonly β1-selective blockers are commonly used to treat cardiovascular disorders and by athletes as doping substances. Several observational studies have reported conflicting data about the association of treatment with beta blockers and the risk of fracture.[50–52] Our observations in mice now provide a potential mechanisms to explain the lower bone mass and increased bone fragility in absence of β1-adrenergic signaling, which apparently cannot be compensated by the antiresorptive effects of β2 blockade.

In conclusion, β1- and β2-adrenergic pathways exert opposite effects on bone. Whereas β1-adrenergic signaling regulates bone anabolic responses during growth and in response to loading, at least in part through systemic mechanisms implicating IGF-1, β2-adrenergic signaling independently regulates bone remodeling through RANKL expression in osteoblasts.

Disclosures

All authors state that they have no conflicts of interest

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