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Abaloparatide, a novel osteoanabolic PTHrP analog, increases cortical and trabecular bone mass and architecture in orchiectomized rats by increasing bone formation without increasing bone resorption☆

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ABSTRACT

Male osteoporosis can occur with advanced age and with hypogonadism, with increased bone resorption and/or inadequate bone formation contributing to reduced bone mass and increased fracture risk. Abaloparatide is a selective PTH receptor agonist that increases bone formation and bone mass in postmenopausal women with osteoporosis and in estrogen-deficient animals. The current study evaluated the effects of abaloparatide in orchiectomized (ORX) rats, a model of male osteoporosis. Four-month-old Sprague-Dawley rats underwent ORX or sham surgery; 8 weeks later the ORX groups exhibited relative osteopenia vs sham controls, based on dual X-ray absorptiometry (DXA) and/or peripheral quantitative computed tomography (pQCT) assessments at the total body, lumbar spine, femur, and tibia. ORX rats (n = 10/group) were then injected daily (s.c.) for 8 weeks with vehicle or abaloparatide at 5 (ABL5) or 25 μg/kg/d (ABL25). Sham controls (n = 10) received s.c. vehicle. DXA and pQCT showed that one or both abaloparatide groups gained more areal and volumetric BMD at all sites analyzed compared with vehicle controls, leading to substantial or complete reversal of ORX-induced BMD deficits. pQCT also indicated greater gains in tibial cortical thickness in both abaloparatide groups versus vehicle controls. Tibial bone histomorphometry showed greater trabecular bone formation and bone volume and improved micro-architecture with abaloparatide, with no increase in osteoclasts. Abaloparatide also led to significant improvements in the balance of biochemical bone formation markers versus bone resorption markers, which correlated with BMD changes. These findings suggest that abaloparatide may have therapeutic benefits in men with osteoporosis.

1. Introduction

Male osteoporosis is a disease of reduced bone mass leading to an increased risk of fragility fractures. Male osteoporosis is often related to hypogonadism, including that which results from androgen deprivation therapy in men with prostate cancer [1,2]. Androgen deficiency promotes bone loss directly via reduced bone cell exposure to testosterone [3,4] and indirectly via reduced androgen conversion to estradiol [5,6]. Androgen deficiency frequently leads to increased bone resorption that contributes to cortical and trabecular bone loss and increased fracture risk [1,7–9]. Male osteoporosis can also be idiopathic, which commonly presents with osteoblast dysfunction and impaired bone formation [10,11]. Current therapeutic agents for the treatment of male osteoporosis include several antiresorptive agents and the PTH receptor agonist PTH(1-34) (teriparatide), all of which improve bone mass in this population [12]. However, antiresorptive agents do not reverse deficits in bone formation that may exist in men with osteoporosis, which limits the potential to reverse micro-architectural deterioration. Conversely, teriparatide significantly increases bone formation in men with osteoporosis but it also increases bone resorption [13], which may

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Table 1
Absolute DXA-derived areal BMD (aBMD) and areal BMC (aBMC) values at the end of the 8-week post-surgical bone depletion period, just prior to treatment. Data represent means and SEM, n = 10/group. *$P < 0.05$ vs Sham by one-way ANOVA and Dunnett's test.

	Sham	Veh	ABL	ABL 25
Whole body aBMD (g/cm^2)	0.173 ± 0.002	0.165 ± 0.001*	0.165 ± 0.002*	0.164 ± 0.001*
Whole body aBMC (g)	15.45 ± 0.385	14.27 ± 0.280*	14.32 ± 0.250*	13.95 ± 0.270*
Lumbar spine aBMD (g/cm^2)	0.264 ± 0.006	0.240 ± 0.005*	0.240 ± 0.005*	0.240 ± 0.003*
Lumbar spine aBMC (g)	0.690 ± 0.030	0.586 ± 0.020*	0.596 ± 0.020*	0.582 ± 0.014*
Total femoral aBMD (g/cm^2)	0.326 ± 0.005	0.298 ± 0.004*	0.302 ± 0.003*	0.298 ± 0.003*
Total femoral aBMC (g)	0.668 ± 0.026	0.577 ± 0.019*	0.591 ± 0.014*	0.567 ± 0.013*
Proximal femoral aBMD (g/cm^2)	0.389 ± 0.008	0.343 ± 0.006*	0.353 ± 0.004*	0.344 ± 0.003*
Proximal femoral aBMC (g)	0.235 ± 0.001	0.205 ± 0.008*	0.208 ± 0.006*	0.200 ± 0.004*
Mid-femoral aBMD (g/cm^2)	0.311 ± 0.008	0.287 ± 0.006	0.291 ± 0.004*	0.284 ± 0.004*
Mid-femoral aBMC (g)	0.137 ± 0.005	0.124 ± 0.005	0.125 ± 0.004	0.118 ± 0.003*
Distal femoral aBMD (g/cm^2)	0.338 ± 0.005	0.311 ± 0.005*	0.309 ± 0.005*	0.314 ± 0.005*
Distal femoral aBMC (g)	0.184 ± 0.006	0.163 ± 0.005*	0.165 ± 0.004*	0.165 ± 0.005*

ABL 5, abaloparatide 5 μg/kg/d; ABL 25, abaloparatide 25 5 μg/kg/d; aBMD, areal bone mineral density; aBMC, areal bone mineral content.

Table 2
Absolute pQCT-derived volumetric BMD (vBMD), volumetric BMC (vBMC), cortical area and cortical thickness data for the right tibial proximal metaphysis or mid-diaphysis at the end of the 8-week post-surgical bone depletion period, just prior to treatment. Data represent means and SEM. *$P < 0.05$ vs Sham by one-way ANOVA and Dunnett's test.

	Sham	Veh	ABL 5	ABL 25
Proximal metaphyseal total vBMD (mg/cm^3)	544.8 ± 14.53	544.61 ± 14.84	519.6 ± 14.80	526.9 ± 14.42
Proximal metaphyseal total vBMC (mg/mm)	11.99 ± 0.37	10.63 ± 0.29*	10.27 ± 0.21*	10.70 ± 0.25*
Proximal metaphyseal trabecular vBMD (mg/cm^3)	234.3 ± 13.0	182.7 ± 16.1*	153.5 ± 12.4*	183.6 ± 15.1*
Proximal metaphyseal trabecular vBMC (mg/mm)	2.07 ± 0.13	1.42 ± 0.11*	1.21 ± 0.08*	1.50 ± 0.12*
Proximal metaphyseal cortical/subcortical vBMD (mg/cm^3)	752.4 ± 18.91	786.3 ± 19.90	764.2 ± 18.8	757.0 ± 16.9
Proximal metaphyseal cortical/subcortical vBMC (mg/mm)	9.92 ± 0.28	9.21 ± 0.26	9.06 ± 0.17*	9.20 ± 0.17
Diaphyseal cortical vBMD (mg/cm^3)	1232.8 ± 5.5	1241.9 ± 4.8	1239.5 ± 5.4	1229.0 ± 4.7
Diaphyseal cortical vBMC (mg/mm)	8.67 ± 0.26	8.11 ± 0.17	8.01 ± 0.18	8.02 ± 0.23
Diaphyseal cortical area (mm^2)	7.03 ± 0.20	6.53 ± 0.14	6.47 ± 0.15	6.53 ± 0.17
Diaphyseal cortical thickness (mm)	0.90 ± 0.02	0.89 ± 0.01	0.88 ± 0.02	0.89 ± 0.02
Diaphyseal periosteal circumference (mm)	10.66 ± 0.16	10.13 ± 0.15*	10.13 ± 0.12*	10.12 ± 0.14*
Diaphyseal endocortical circumference (mm)	5.04 ± 0.15	4.52 ± 0.16*	4.63 ± 0.13	4.51 ± 0.13*

ABL 5, abaloparatide 5 μg/kg/d; ABL 25, abaloparatide 25 5 μg/kg/d; vBMD, volumetric bone mineral density; vBMC, volumetric bone mineral content.

limit its bone-building potential.

Abaloparatide (ABL) is an osteoanabolic 34-amino-acid analog of the amino-terminal (1-34) fragment of human parathyroid hormone-related peptide (PTHrP) [14]. Abaloparatide reduces fracture risk in postmenopausal women with osteoporosis by increasing bone formation and bone mineral density (BMD) with minimal increases in bone resorption [15,16]. Abaloparatide was shown to increase BMD and bone strength in estrogen-deficient ovariectomized (OVX) rats and cynomolgus monkeys by increasing bone formation without increasing bone resorption [17–19]. The minimal effects of abaloparatide on bone resorption may relate in part to lesser increases in the pro-resorptive cytokine RANKL (receptor activator of nuclear factor kappa B ligand) compared with the effect of teriparatide [20,21]. This difference may be relevant in androgen-deficient states because ORX rat studies indicate that androgen deficiency increases RANKL levels in a manner that promotes bone resorption and bone loss [22,23]. Based on evidence that androgen deficiency leads to estrogen deficiency [5,6], and that abaloparatide increases bone formation and bone mass in estrogen-deficient osteopenic and osteoporotic states, we hypothesized that abaloparatide would also have skeletal benefits in androgen-deficient osteopenic states.

Orchiectomized (ORX) rats have combined androgen and estrogen deficiency [24] and often exhibit increased bone resorption [23,24] and decreased periosteal bone formation [22]. These changes have adverse effects on bone mass and geometry that mimic some of the changes observed in men with androgen deficiency [25–27]. Abaloparatide was previously shown to increase trabecular and periosteal bone formation in estrogen-deficient rats [19], a profile that seems well-suited to improve skeletal mass and structure in ORX rats. A preliminary ORX rat study showed that 4 weeks of daily abaloparatide administration at a single dose level increased vertebral BMD and improved trabecular bone volume and architecture [28]. The current study assessed the effects of two abaloparatide dose levels over an 8 week treatment period on cortical and trabecular bone densitometry variables, trabecular histomorphometry, and bone turnover markers in osteopenic ORX rats.

2. Materials and methods

2.1. Animals and animal care

All animal procedures and activities were approved by Charles River Montreal's Institutional Animal Care and Use Committee and performed in an AAALAC-accredited facility (Charles River Laboratories, Montreal, Canada). Thirty ORX male Sprague-Dawley rats and 10 sham-operated controls were received from Charles River Canada (St. Constant, Quebec) at 4 months of age and were cared for in accordance with established guidelines [29]. Rats were group-housed (up to 3 rats of the same dosing group) in polycarbonate cages containing appropriate bedding and automated watering. Vivarium conditions included a 12-h light/dark cycle, a temperature range of 19 °C to 25 °C, and a relative humidity range of 30% to 70%. Animals received Certified Rodent Chow No. 5CR4 (PMI Nutrition International) ad libitum and filtered UV-irradiated municipal tap water via an automatic watering system. Animals were observed for general health twice daily and underwent detailed clinical observations weekly. Food consumption and body weights were monitored regularly throughout the study.

2.2. Study design and abaloparatide dose selection

After surgery, rats were left untreated for 8 weeks to allow the

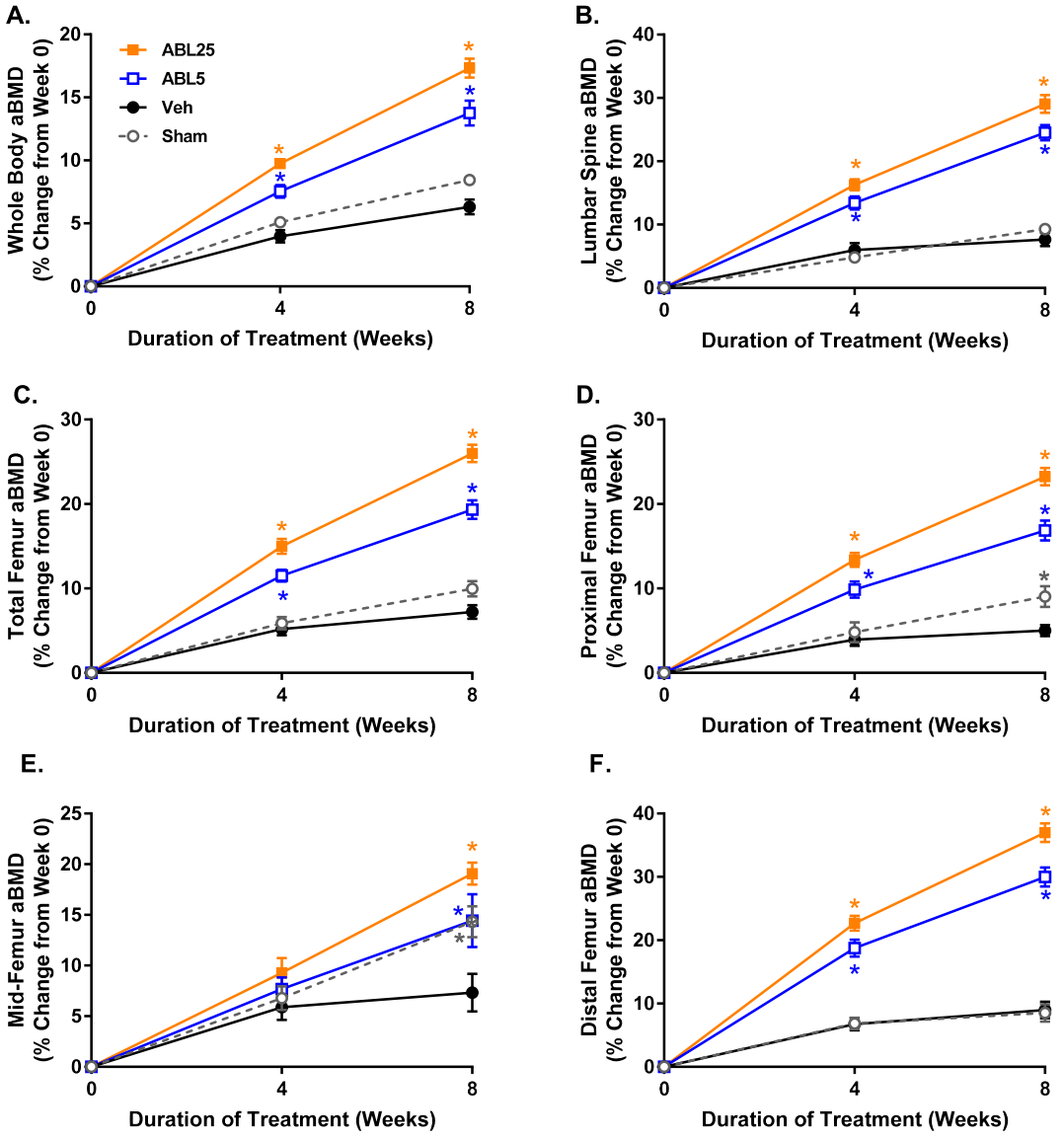

Fig. 1. In vivo DXA-derived areal bone mineral density (aBMD) expressed as percentage change from Week 0 (treatment baseline) through the end of treatment. A) whole body aBMD; B) lumbar spine aBMD; C) total femur aBMD; D) proximal femur aBMD; E) mid-femur diaphysis aBMD; F) distal femur aBMD. Veh, vehicle; ABL5, abaloparatide 5 μg/kg/d; ABL25, abaloparatide 25 μg/kg/d. Data represent mean and SEM, n = 10/group. *$P < 0.05$ vs Veh control.

development of relative osteopenia after ORX. The ORX rats were assigned to 3 groups of 10 animals each using a randomization scheme that minimized inter-group differences in body weight and lumbar spine areal BMD (aBMD), which was assessed at the end of the bone depletion period as described below. The ORX groups then received vehicle (Veh; 0.9% sodium chloride) or abaloparatide at 5 (ABL5) or 25 (ABL25) μg/kg/d by daily s.c. injection for 8 weeks. Sham controls (n = 10) received daily s.c. vehicle. Abaloparatide dose levels were selected based on previous studies in female estrogen-deficient rats showing that these doses were well tolerated and caused significant increases in bone formation, BMD, and bone strength [18,19]. Rats were euthanized at the end of the 8-week treatment period by exsanguination from the abdominal aorta under isoflurane anesthesia. All animals received s.c. injections of calcein green (8 mg/kg) on the 10th and 3rd day prior to sacrifice to fluorescently label bones for dynamic histomorphometry.

2.3. Bone densitometry

In vivo dual X-ray absorptiometry (DXA) was performed on the whole body, right femur, and lumbar spine (L1 to L4) of anaesthetized rats using a Discovery A bone densitometer (Hologic Inc., Bedford, MA, USA). Single scan measures of bone area, aBMD, and areal bone mineral content (aBMC) were obtained prior to initiating treatments (i.e., week 0) and again at weeks 4 and 8 of the treatment period. In vivo peripheral quantitative computed tomography (pQCT) was performed on the right tibia of anaesthetized rats with an XCT Research SA+ bone scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany) prior to initiating treatments (week 0) and again at weeks 4 and 8 of treatment. With pQCT, a single slice (0.15 mm anisotropic voxel size) was obtained at the right proximal tibial metaphysis (9% of tibial length from the proximal end) and another at the mid-diaphysis (50% of tibial length). The tibial metaphysis was segmented for pQCT into trabecular and cortical/subcortical compartments, with the total region comprising both compartments combined. Metaphyseal settings were Contmode 2, Peelmode 20, and trabecular area of 40%; diaphyseal settings were Contmode 2, Cortmode 2, and a standard threshold of 570 mg/cm^3.

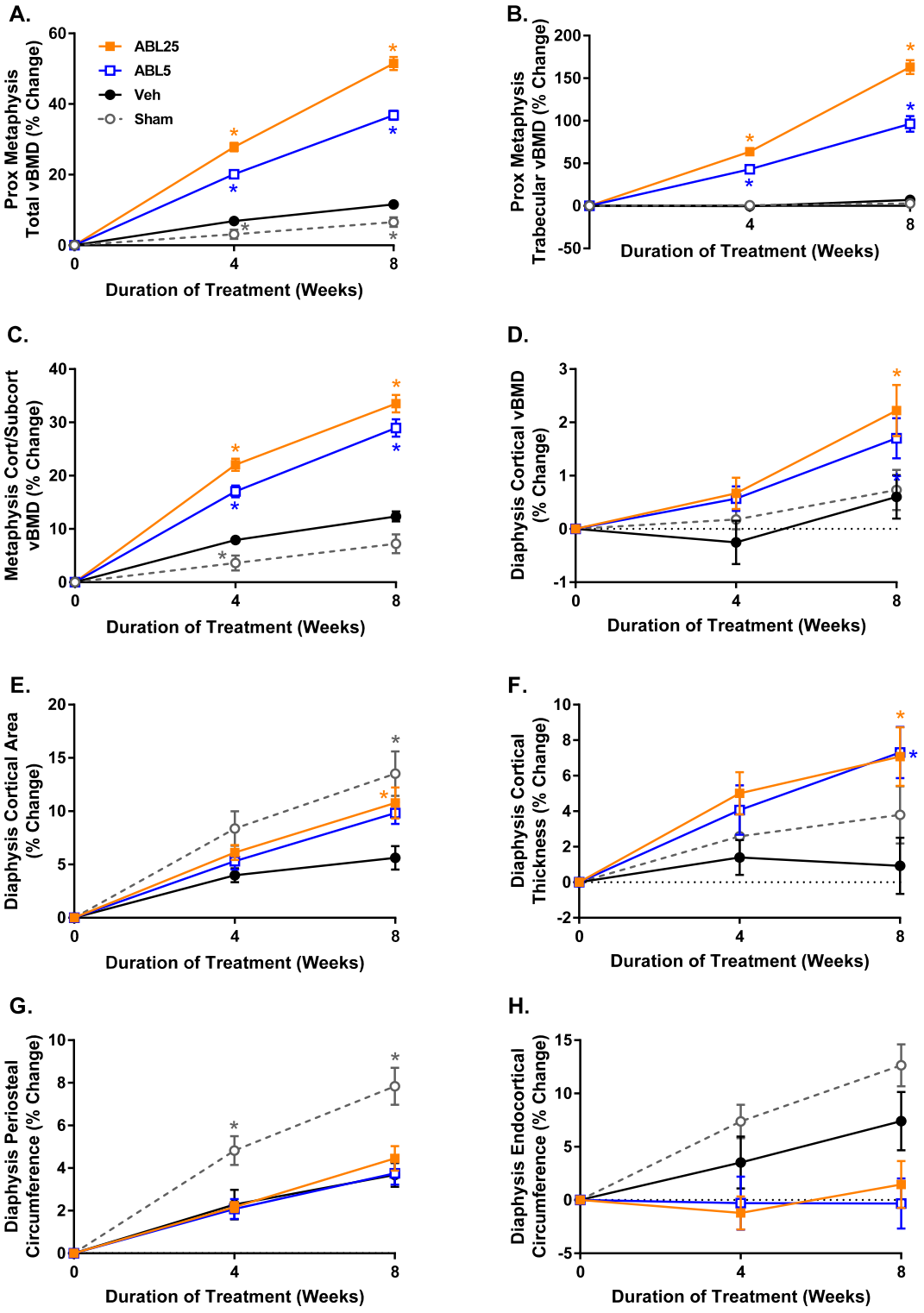

Fig. 2. In vivo pQCT parameters for the tibia, expressed as percentage change from Week 0 (treatment baseline) through the end of treatment. A) proximal metaphysis total (cortical plus trabecular) volumetric BMD (vBMD); B) proximal metaphysis trabecular vBMD; C) metaphysis cortical/subcortical vBMD; D) diaphysis cortical vBMD; E) diaphysis cortical area; F) diaphysis cortical thickness; G) diaphysis periosteal circumference; H) diaphysis endocortical circumference. Veh, vehicle; ABL5, abaloparatide 5 μg/kg/d; ABL25, abaloparatide 25 μg/kg/d. Data represent means and SEM, n = 10/group. *$P < 0.05$ vs Veh control.

2.4. Bone histomorphometry

The right tibia was collected at necropsy, and tibiae from 8 randomly-selected animals of each group were processed for histomorphometry. Tibiae were formalin-fixed for 3 days and transferred to 70% ethanol. They were then cut with a handsaw to obtain a proximal end with a sagittal face and further dehydrated with graded ethanol, cleared with xylene, and embedded undecalcified in methylmethacrylate. Consecutive longitudinal sections of the proximal tibia were cut at 5-micron thickness with an RM2255 microtome (Leica, Germany). Two serial sections were stained with 2% Toluidine blue (pH 3.7) for osteoid and cellular parameters, data for which were averaged. Another section

Table 3
Cancellous bone histomorphometry at the proximal tibial metaphysis at the end of the 8-week treatment period. Data represent means and SEM, n = 8/group. *$P < 0.05$ vs. Veh control.

	Sham	Veh	ABL 5	ABL 25
BV/TV (%)	14.7 ± 1.1	13.5 ± 2.9	23.2 ± 2.9	41.9 ± 4.2*
Tb.Th (μm)	66.0 ± 3.5	81.5 ± 5.1	110.3 ± 7.9*	116.2 ± 10.9*
Tb.N (mm^{-1})	2.26 ± 0.19	1.59 ± 0.27	1.99 ± 0.27	2.56 ± 0.15
Tb.Sp (μm)	396.8 ± 31.8*	698.5 ± 146.4	362.0 ± 34.2*	250.7 ± 32.0*
MAR (μm/d)	1.65 ± 0.08	1.66 ± 0.05	1.67 ± 0.27	1.86 ± 0.07
MS/BS (%)	32.4 ± 1.4	39.1 ± 2.02	45.9 ± 6.8	58.6 ± 1.3*
BFR/BS ($\mu m^3/\mu m^2$/d)	0.54 ± 0.04	0.65 ± 0.04	0.88 ± 0.14	1.09 ± 0.03*
OS/BS (%)	4.18 ± 0.67	8.96 ± 2.45	5.42 ± 1.01	5.14 ± 1.38
O.Th (μm)	5.86 ± 0.18	5.45 ± 0.37	5.86 ± 0.23	6.46 ± 0.17
Oc.S/BS (%)	7.50 ± 0.62	9.72 ± 0.89	8.70 ± 1.49	7.15 ± 2.53

BV, bone volume; TV, total volume; Tb, trabecular; Th, thickness; N, number; Sp, separation; MAR, mineral apposition rate; MS, mineralizing surface; BS, bone surface; BFR, bone formation rate; OS, osteoid surface; O, osteoid; Oc.S, osteoclast surface.

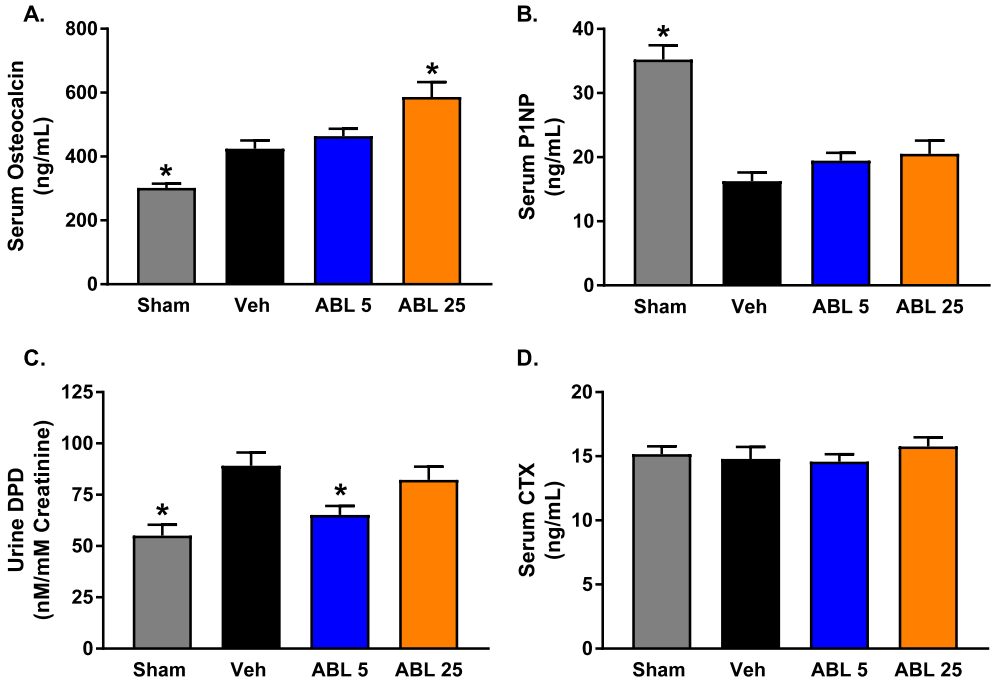

Fig. 3. Biochemical markers of bone formation (A, serum osteocalcin; B, serum P1NP) and bone resorption (C, urine DPD and D, serum CTX) at the end of the 8-week treatment period. Data represent means and SEM, n = 10/group. *$P < 0.05$ vs Veh control.

was mounted unstained for the assessment of fluorescence-based dynamic parameters. Sections were analyzed with a Nikon E800 microscope equipped with an Olympus DP71 digital camera that captured images via Olympus CellSens software. Data were obtained at 100× magnification from a 0.9 mm by 1.3 mm region away from the proximal growth plate using OsteoMeasure software (Osteometrics Inc., Decatur, GA) and standardized bone histomorphometry analyses and nomenclature [30].

2.5. Biochemical markers of bone turnover

Approximately 1.5 mL of whole blood was collected in the early morning from the jugular vein of overnight-fasted rats at the end of the 8-week treatment period. Serum was prepared and stored at −20 °C until use. Thawed serum was analyzed by enzyme-linked immunosorbent assay (ELISA) kits for procollagen type I N-telopeptide (P1NP; IDS, Gaithersburg, MD, USA), osteocalcin (OC; Biomedical Technologies Inc., Madrid, Spain), and C-telopeptides of type I collagen (CTX; IDS, Gaithersburg, MD, USA). Urine collected from rats fasted overnight in metabolism cages was stored at −20 °C, and thawed urine was analyzed for deoxypyrodinoline (DPD) by an ELISA kit (Quidel, San Diego, CA, USA), with DPD values adjusted for urine creatinine levels.

Bone turnover markers for the Veh, ABL5 and ABL25 groups were combined for linear regression analyses versus percentages change in lumbar spine aBMD. As an exploratory analysis, bone turnover markers were also used to calculate anabolic windows that reflect the relative balance of bone formation and bone resorption with or without abaloparatide treatment [19]. To calculate anabolic windows, bone turnover marker data were first normalized by dividing marker values of individual animals from the Veh, ABL5, and ABL25 groups by the average value of the Veh control group. Normalized values for the resorption markers (CTX and DPD) were then subtracted from normalized values for the formation markers (P1NP or osteocalcin), with differences reflecting the extent to which treatment-related responses of bone formation markers exceeded responses of bone resorption markers.

2.6. Statistics

Percentage changes from treatment baseline in DXA and pQCT parameters were analyzed with Levene's test to assess the homogeneity of group variances. If group variances were similar by Levene's test a one-way ANOVA was applied, followed when appropriate by Dunnett's

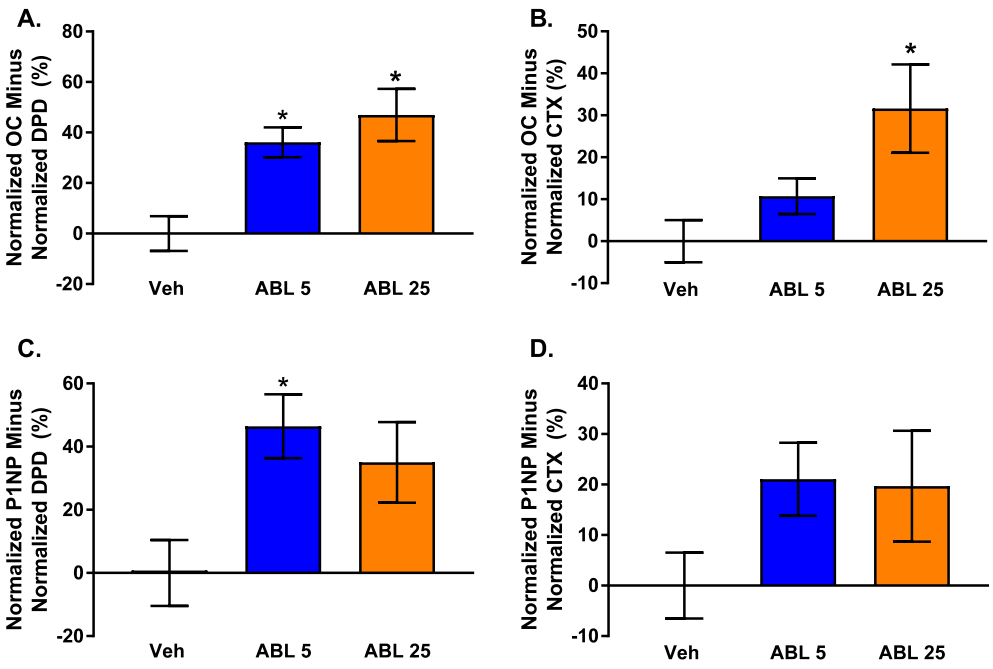

Fig. 4. Anabolic windows as determined from biochemical markers of bone turnover measured at the end of the 8-week treatment period. Serum P1NP, osteocalcin (OC), CTX, and urine DPD data for all three ORX groups were normalized by expressing the biomarker values for each animal as a percentage of the respective biomarker's mean value from the Veh control group. Anabolic windows were then calculated by subtracting normalized serum CTX or urine DPD values from normalized serum OC or P1NP value. A) normalized osteocalcin – normalized DPD (% Veh control); B) normalized osteocalcin – normalized CTX (% Veh control); C) normalized P1NP – normalized DPD (% Veh control); D) normalized P1NP – normalized CTX (% Veh control). Data represent means and SEM, n = 10/group. *$P < 0.05$ vs Veh controls.

post-test to compare the Veh group with each other group. If Levene's test showed unequal variances Kruskal-Wallis tests were applied, followed when appropriate by Dunn's post-test to compare the Veh group with each other group. These tests were performed using Charles River Labs' in-house SAS-based statistics system (SAS Institute Inc., Cary, NC, USA).

Absolute DXA and pQCT data obtained at the beginning (Week 0) and end (Week 8) of the treatment period were analyzed by one-way ANOVA; for Week 0 results, Dunnett's post-test was used to compared Sham controls to the other 3 groups, and for Week 8 results Dunnett's post-test was used to compare Veh controls to the other three groups. Bone turnover marker and bone histomorphometry data were analyzed by one-way ANOVA followed by Dunnett's tests comparing Veh controls to the other three groups. Anabolic windows were analyzed by one-way ANOVA followed by Dunnett's post-test, with each abaloparatide group compared with Veh controls. These tests and all linear regression analyses were performed using Prism GraphPad V6. For all statistical tests, a P value of < 0.05 was used to indicate significant differences.

3. Results

3.1. General health

All study animals survived in good health until scheduled necropsy at the end of the 8-week treatment period. Success of ORX surgery was confirmed by the absence of testes and epididymis at necropsy and by a significant 10.4% deficit in body weight gain in the ORX groups compared with Sham controls at the treatment baseline (data not shown). Abaloparatide was well-tolerated and its administration had no effect on body weight gain or food consumption compared with Veh controls (data not shown). No visible skeletal lesions were observed in any animal at necropsy.

3.2. Bone densitometry

The 8-week post-surgical bone depletion period led to significant ORX-related deficits in bone mass, density, and geometry. DXA assessments at the treatment baseline (week 0) revealed that the groups designated for Veh, ABL5 and ABL25 treatment had significantly lower aBMD and aBMC for the whole body, lumbar spine, total femur, proximal femur, and distal femur compared with Sham controls; some but not all of the ORX groups also exhibited significantly lower mid-femoral aBMC and aBMD compared with Sham controls (Table 1). pQCT performed at the treatment baseline showed that the proximal tibial metaphysis of the Veh, ABL5, and ABL25 groups had significantly lower trabecular volumetric BMD (vBMD) and significantly lower total and trabecular volumetric BMC (vBMC) compared with sham controls (Table 2). The Veh, ABL5, and ABL25 groups also had significantly lower tibial diaphyseal periosteal circumferences at the treatment baseline compared with sham controls, and the Veh and ABL25 groups had significantly lower endocortical circumference versus sham controls. Tibial diaphyseal cortical vBMD, vBMC, area, and thickness were similar in the three ORX groups and sham controls at the treatment baseline (Table 2).

DXA assessments showed that the ABL5 and ABL25 groups experienced significantly greater aBMD gains from the treatment baseline to treatment week 8 for the whole body, lumbar spine, total femur, proximal femur, mid-femur, and distal femur compared with Veh controls (Fig. 1). Abaloparatide-related aBMD gains for each of these sites except the mid-femur achieved significance versus Veh by week 4 of treatment. By the end of the treatment period, all DXA regions for the abaloparatide groups showed substantial or complete recovery of aBMD and aBMC values toward or above the level of sham controls, and final DXA values in both abaloparatide groups significantly exceeded Veh controls except for mid-femoral aBMC (Supplemental Table 1).

pQCT showed that the proximal tibial metaphysis of the ABL5 and ABL25 groups had significantly greater increases from treatment baseline in total vBMD, trabecular vBMD, and cortical/subcortical vBMD after 4 and 8 weeks of treatment compared with Veh controls (Fig. 2A–C). The tibial diaphysis of the ABL25 groups also showed significantly greater gains in cortical vBMD at week 8 compared with Veh controls (Fig. 2D). Cortical area and thickness of the tibial diaphysis showed greater increases in the ABL25 group at week 8, and cortical thickness was also increased in the ABL5 group at week 8, compared with Veh controls (Fig. 2E–F). The Veh, ABL5, and ABL25 groups showed similar gains in periosteal circumference of the tibial diaphysis during the treatment period, whereas sham controls exhibited greater periosteal expansion compared with Veh controls (Fig. 2G). The sham and Veh groups showed increases from treatment baseline in endocortical circumference, whereas endocortical circumference did

not increase in the ABL5 or ABL25 groups (Fig. 2H). At the end of the treatment period, the proximal tibial metaphysis of the ABL5 and ABL25 groups showed recovery of total, trabecular, and cortical/subcortical vBMD and vBMC to levels that statistically exceeded Veh controls and numerically exceeded sham controls (Supplemental Table 2). Absolute diaphyseal cortical thickness in the ABL25 showed partial recovery relative to sham controls, with levels significantly exceeding Veh control values (Supplemental Table 2).

3.3. Bone histomorphometry

Histomorphometry at the proximal tibial metaphysis indicated greater trabecular bone volume and improved microarchitecture in one or both abaloparatide groups compared with Veh controls (Table 3). Trabecular BV/TV and trabecular thickness (Tb.Th) were greater in the ABL25 group and Tb.Th was also greater in the ABL5 group compared with Veh controls. Trabecular spacing (Tb.Sp), which was nearly two-fold higher in the Veh vs Sham group, was markedly lower in both abaloparatide groups compared with Veh. The ABL25 group showed significantly greater trabecular mineralizing surface (MS/BS) and bone formation rate (BFR/BS) vs Veh controls. No differences were observed between the four groups for mineral apposition rate (MAR), osteoid surface (OS/BS), osteoid thickness (O.Th), or osteoclast surface (Oc.S/BS).

3.4. Bone turnover markers

At the end of the treatment period the bone formation marker serum osteocalcin was significantly higher in the ABL25 group compared with Veh controls, whereas serum P1NP showed minor and non-significant increases in the ABL5 and ABL25 groups compared with Veh controls (Fig. 3A–B). The resorption marker urine DPD was significantly lower in the ABL5 group and similar in the ABL25 group compared with Veh controls (Fig. 3C), and the resorption marker serum CTX was similar in all four groups (Fig. 3D). Linear regression analyses of week 8 bone turnover marker values for the three ORX groups versus the percentage change in lumbar spine aBMD during the treatment period showed significant positive relationships for P1NP ($r = 0.41$; $P < 0.05$) and osteocalcin ($r = 0.53$; $P < 0.005$), and no significant relationships for CTX or DPD.

Bone turnover markers were also used to calculate anabolic windows, which reflect the numerical extent to which bone formation marker responses to ABL exceed bone resorption marker responses. Significant anabolic windows were observed for the osteocalcin vs DPD response in the ABL5 and ABL25 group, and for the osteocalcin vs CTX response in the ABL25 group (Fig. 4A–B). A significant anabolic window was also evident for the P1NP vs DPD response in the ABL5 group (Fig. 4C), whereas the window for the P1NP vs CTX response did not achieve significance for either abaloparatide group (Fig. 4C). Linear regression analyses of anabolic windows versus percentage change in lumbar spine aBMD during treatment indicated significant positive correlations in all cases, with r values of 0.68 for OC-DPD, 0.51 for OC-CTX, 0.48 for P1NP-DPD, and 0.38 for P1NP-CTX (all $P < 0.005$).

4. Discussion

This study shows for the first time that abaloparatide increases bone formation and improves cortical and trabecular bone mass, BMD, geometry, and microarchitecture in an osteopenic state driven by androgen deficiency. ORX rats receiving abaloparatide exhibited substantial or complete BMD restoration at the whole body, lumbar spine, total femur and the femoral diaphysis, with positive effects evident in both cortical and trabecular bone compartments. Cortical bone in abaloparatide-treated rats showed relatively greater vBMD, area, and thickness, with the latter two effects attributable to ongoing periosteal expansion in conjunction with the prevention of endocortical expansion. Trabecular bone in abaloparatide-treated rats showed substantial gains in vBMD and BV/TV and improved trabecular micro-architecture. Trabecular bone gains are an important therapeutic goal for men with osteoporosis, many of whom experience progressive bone loss at trabecular-rich sites for decades prior to their initial osteoporosis diagnosis or first fragility fracture [31]. Cortical bone gains are also likely to benefit men with osteoporosis, many of whom experience substantial cortical bone loss at older ages that further contributes to increased fracture risk [31,32].

Histomorphometry indicated that the higher abaloparatide dose significantly increased trabecular bone formation rate and the bone formation marker serum osteocalcin. Neither abaloparatide group exhibited increased trabecular osteoclast surface compared with Veh controls, nor did they show increases in the bone resorption markers serum CTX or urine DPD; similar findings as these were observed in female OVX rats treated with these same abaloparatide doses over a 12-month period [19]. Evidence for increased bone formation and BMD with abaloparatide without increases in bone resorption indices is aligned with and extends previous clinical and preclinical data indicating that BMD gains with PTH receptor agonists do not necessarily require increased bone resorption; indeed, the pro-resorptive responses to teriparatide appear to limit its bone-building potential [33,34]. When the pro-resorptive effect of PTH receptor activation is minimized or prevented, substantial gains in bone mass may be achievable with only modest increments in bone formation, presumably due to a more favorable bone balance [33]. Changes in bone balance were indirectly assessed in the current study by calculating anabolic windows, which reflected the extent to which abaloparatide-induced increments in the formation markers P1NP or osteocalcin exceeded increments in the resorption markers CTX or DPD. Significant anabolic windows in one or both abaloparatide groups were evident for the osteocalcin vs DPD response, the osteocalcin vs CTX response, and the P1NP vs DPD response. These anabolic windows correlated positively with treatment-related BMD changes, and they appeared to explain more of the variation in aBMD change compared with that explained by individual bone turnover markers. Similar anabolic window findings were observed in abaloparatide-treated ovariectomized rats [19], suggesting that abaloparatide-related gains in bone mass in gonadectomized male and female rats are largely achieved through a more favorable bone balance.

This study has several limitations, some of which relate to the animal model and its clinical generalizability. The vehicle-treated ORX rats exhibited deficits in periosteal and endocortical dimensions, perhaps due to lesser androgen-mediated stimulation of radial bone growth, whereas the long bones of older men with osteoporosis tend to exhibit increased periosteal and endocortical dimensions [32], due perhaps to normal aging or hypogonadism-related reductions in estradiol, a hormone that normally restrains the radial expansion of long bones [35]. As with all ORX models, the abruptness of sex hormone ablation with castration may not faithfully mimic the effects of gradual age-related hypogonadism on bone. Another limitation is that bone histomorphometry was assessed at end but not at the beginning of treatment, making it difficult to know the extent to which more robust trabecular microarchitecture in the abaloparatide groups was due to reversal versus prevention of ORX-induced microarchitectural deterioration. Finally, bone strength was not evaluated and is an important endpoint for future analyses based on its relevance as a surrogate for fracture risk reduction.

In summary, abaloparatide effects were studied in ORX rats to evaluate its potential as an investigational therapy for men with osteoporosis. New approaches for treating male osteoporosis may help to address major treatment gaps that currently exist. Around 40% of all fragility fractures happen in men [8], and mortality after an osteoporotic fracture is higher for men than for women [36], yet osteoporosis diagnosis rates in men are remarkably low, and < 10% of men with a new fragility fracture are prescribed an osteoporosis medication [37]. Male osteoporosis frequently develops through elevated bone

resorption and bone formation that is either suppressed or insufficiently increased to counter higher bone resorption [6,38]. Abaloparatide stimulates bone formation and improves cortical and trabecular BMD, geometry, and architecture in ORX rats without increasing parameters of bone resorption, suggesting that abaloparatide holds promise as a potential therapy for male osteoporosis.

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Appendix A. Supplementary data

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