Pharmaceutical Research, Vol. 26, No. 11, November 2009 (© 2009)
DOI: 10.1007/s11095-009-9962-7

Research Paper

Effects of Selective Androgen Receptor Modulator (SARM) Treatment in Osteopenic Female Rats

Jeffrey D. Kearbey,[1,2] Wenqing Gao,[1] Scott J. Fisher,[1] Di Wu,[1] Duane D. Miller,[2,3] and James T. Dalton[1,2,4]

Received July 1, 2009; accepted August 20, 2009; published online September 1, 2009

Purpose. Although androgens are known to protect bone, side effects and poor oral bioavailability have limited their use. We previously reported that S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide (S-4) is a potent and tissue-selective androgen receptor modulator (SARM). This study was designed to evaluate the skeletal effects of S-4 in an osteopenic model.
Methods. Aged female rats were gonadectomized or sham operated on day 1 and assigned to treatment groups. Dosing was initiated on day 90 and continued daily until day 210. Whole animal bone mineral density (BMD), body weight, and fat mass were determined by dual energy x-ray absorptiometry (DEXA). Regional analysis of excised bones was performed using DEXA or computed tomography. Femur strength was evaluated by 3-point bending.
Results. S-4 restored whole body and lumbar vertebrae (L5-L6) BMD to the level of intact controls. Significant increases in cortical bone quality were observed at the femoral midshaft, resulting in increased load bearing capacity.
Conclusions. S-4 demonstrated partial/complete recovery of bone parameters to age-matched intact levels. Increased efficacy observed in cortical bone sites is consistent with reported androgen action in bone. The ability of S-4 to promote bone anabolism, prevent bone resorption, and increase skeletal muscle mass/strength positions these drugs as promising new alternatives for the treatment of osteoporosis.

KEY WORDS: androgens & SARMs; bone densitometry; bone QCT; mechanical loading; rodent.

INTRODUCTION

Osteoporosis is a major clinical problem in elderly populations, and the incidence increases with age. Since a primary reason for diagnosis of osteoporosis is a fracture, it is considered a "silent" disease. Therapeutic intervention is often not begun until after a significant amount of bone has already been lost. Unfortunately, the majority of current treatments focus on preventing resorption of existing bone (1). The effects of estrogen, selective estrogen receptor modulators (SERMs), and bisphosphonates are mediated by inhibition of bone loss and corresponding reduction of bone turnover. This action results in modest increases in bone mass since resorption is inhibited while bone formation is not altered (1). Currently, there is a clinical void for agents that have anabolic actions in bone. Agents which act through stimulation of the osteoblasts have the potential to increase bone density to a much greater degree than anti-resorptive therapies. Thus, anabolic therapies for osteoporosis would offer many significant advantages over currently available options. Fluoride, growth hormone, insulin-like growth factor, strontium, statins, tibolone, and parathyroid hormone (PTH) have all been examined for anabolic skeletal effects. Currently, PTH is the most promising, but many questions regarding anabolic effects on cortical bone, fracture risk reduction, and development of drug delivery systems remain to be answered (1).

There is increasing evidence to support an anabolic role for androgens in bone (2–5). Studies by Hanada *et al.* (3) showed that a tetrahydroquinoline-derived SARM (S-40503) exhibits anabolic effects in an orchidectomized rat model of bone loss. They showed that S-40503 was able to maintain cancellous bone in a gonadectomized male rat, but not in an ovariectomized female model. However, S-40503 increased cortical BMD in both models. These studies suggest that SARMs can modulate bone mass. Additionally, previous studies in our laboratory have shown that S-4 prevents bone loss in acutely ovariectomized (OVX) female rats (6). We observed increases in whole-body BMD, regional BMD,

Funding Sources Supported by a grant from GTx, Inc., Memphis, TN.

[1] College of Pharmacy, Division of Pharmaceutics, The Ohio State University, Columbus, Ohio 43210, USA.
[2] GTx, Inc., 3 N. Dunlap St., Memphis, Tennessee 38163, USA.
[3] Graduate Health Sciences Center, College of Pharmacy, Department of Pharmaceutical Sciences, University of Tennessee, Memphis, Tennessee 38163, USA.
[4] To whom correspondence should be addressed. (e-mail: jdalton@gtxinc.com)

cortical thickness (CT), and trabecular density (TD) with S-4 treatment following ovariectomy.

Although androgens show therapeutic promise in bone, aromatization, virilizing side effects, and lack of adequate dosage formulations have slowed the development of steroidal androgen therapies for osteoporosis. S-4 has previously demonstrated tissue-selective anabolism in the Hershberger Assay by restoring the levator ani muscle (indicator of anabolic activity) to 100% of intact controls, while the prostate (indicator of androgenic activity) was only restored to 36% of intact controls at a dose of 1 mg/day in castrated male rats (7). S-4 binds tightly and specifically to the androgen receptor (AR) (7), is orally bioavailable (8), and is not converted to estrogenic metabolites. These properties make S-4 an ideal pharmacologic tool to explore the effects of androgens in bone. We hypothesized that a nonsteroidal SARM would stimulate bone apposition in the skeleton of chronically ovariectomized (i.e., osteopenic) rats. To this end, we examined the therapeutic effects of S-4 on both cortical and cancellous bone in osteopenic female rats. It is important to note that the study presented herein represents a major paradigm change from our previous study. When animals are treated immediately after ovariectomy as previously reported (6), the result is an assessment of the ability of the drug to prevent bone resorption or suppress bone turnover (similar to the effects of bisphosphonates and SERMs). By delaying treatment for 90 days (as in the current study), the result is an assessment of the ability of the drug to build or restore bone that has already been lost (i.e., bone anabolism).

MATERIALS AND METHODS

One-hundred-twenty female Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). The animals were housed three per cage and were allowed free access to tap water and commercial rat chow (Harlan Teklad 22/5 rodent diet – 8640). During the course of the study, the animals were maintained on a 12 h light:dark cycle. This study was reviewed and approved by the Institutional Laboratory Care and Use Committee of The Ohio State University. The non-aromatizable androgen, dihydrotestosterone (DHT), was included in both intact and OVX animals to serve as a positive androgen-treated control group. At 23 weeks of age, the animals were OVX or sham-operated and then assigned to one of 12 treatment groups (Table I) of ten animals as follows: (1) OVX+S-4 (0.1 mg/day); (2) OVX+S-4 (0.3 mg/day); (3) OVX+S-4 (0.5 mg/day); (4) OVX+S-4 (0.75 mg/day); (5) OVX+S-4 (1.0 mg/day); (6) OVX+S-4 (3.0 mg/day); (7) OVX+DHT (1 mg/day); (8) OVX+S-4 (0.5 mg/day)+ Antiandrogen (1.0 mg/day); (9) OVX+Vehicle; (10) intact+S-4 (1 mg/day); (11) intact+DHT (1 mg/day); (12) intact+Vehicle. The dose range was chosen based on previous pharmacodynamic studies performed in our laboratory (7). The vehicle control group (Group 9) also served as the vehicle control group for a parallel study in which drug treatment began immediately following OVX and continued for 120 days (6), whereupon the animals were euthanized and samples collected for *ex vivo* analysis. Therefore, we collected DEXA scans at day 90 post ovariectomy, and excised bones were collected at day 120 post ovariectomy in order to generate baseline values for comparison. Wronski *et al.* have reported that there is little change in the bone parameters of OVX animals between days 90 and 120 (9). Four animals died during the course of the study. Therefore, groups 5, 7, 8, and 11 consisted of nine animals each. Dosing solutions were prepared daily by dissolving drug in dimethyl sulfoxide (DMSO) and diluting in polyethylene glycol 300 (PEG 300). Compounds were administered beginning at day 90 and continued for 120 days via daily subcutaneous injections in a volume of 0.20 ml.

Total body bone mineral density (BMD), percent fat mass (FM), body weight (BW), bone mineral content (BMC), bone mineral area (BMA), and lean mass (LM) were determined by dual energy x-ray absorptiometry (DEXA) (GE, Lunar Prodigy™) using the small animal software (Lunar enCORE, version 6.60.041) on days 90 and 210 for all treatment groups. Animal BW was also determined by standard gravimetric methods using a 700 series Ohaus triple beam animal balance (Florham Park, NJ). Animals were anesthetized with ketamine:xylazine (87:13 mg/kg) and placed in a prone position for DEXA scanning. The entire animal was selected as the region of interest during data processing to determine whole body parameters. The parameters determined to be sensitive to estrogen withdrawal (i.e., significant differences observed between intact and OVX control groups) were reported herein.

Immediately following the whole body DEXA scan on day 210, animals were sacrificed, and the lumbar vertebrae, femurs, and tibia were excised and cleared of soft tissue. The excised bones from the OVX control group were analyzed immediately following sacrifice on day 120. A 3 in. deep room temperature water bath was used to simulate soft tissue during the DEXA scans of the excised bones. We analyzed the lumbar vertebrae (L5-L6) and a region of the femur consisting primarily of cortical bone for BMD with the Lunar enCORE small animal software.

The right femurs from the OVX + 1.0 mg/day S-4 (Group 5), OVX + 3.0 mg/day S-4 (Group 6), OVX + 1.0 mg/day DHT (Group 7), OVX control (Group 9), and intact control (Group 12) groups were sent to Skeletech, Inc. (Bothell, WA) for peripheral quantitative computed tomography (pQCT) analysis and biomechanical testing. A Stratec XCT RM and associated software (Stratec Medizintechnik GmbH, Pforzheim, Germany. Software version 5.40 C) were used for the pQCT analysis. The femur was analyzed at both the mid-shaft and distal regions. The mid-shaft analysis was performed on the region at 50% of the length of the femur. The distal analysis was performed on the region at 20% of the length of the femur starting at the distal end. One 0.5 mm slice perpendicular to the long axis of the femur was used for analysis. Total BMC, total BMA, total BMD, cortical bone mineral content, cortical bone area, cortical bone mineral density, cortical thickness, periosteal perimeter (circumference), and endosteal perimeter were determined at the mid-shaft of the femur. At the distal femur, total BMC, total BMA, total BMD, trabecular BMC, trabecular BMA, and trabecular BMD were determined. Following pQCT analysis, the femoral strength was determined by a three-point bending test. The anterior to posterior diameter (APD) (unit:mm) at the midpoint of the femoral shaft was measured with an electronic caliper. The femur was placed on the lower supports of a three-point bending fixture with the anterior

Table I. Summary of Treatment Groups

Group Number	Gonadal Status	S-4 (mg/day)	DHT (mg/day)	Bicalutamide (mg/day)
1	OVX	0.1	–	–
2	OVX	0.3	–	–
3	OVX	0.5	–	–
4	OVX	0.75	–	–
5	OVX	1.0	–	–
6	OVX	3.0	–	–
7	OVX	–	1.0	–
8	OVX	0.5	–	1.0
9	OVX	–	–	–
10	Intact	1.0	–	–
11	Intact	–	1.0	–
12	Intact	–	–	–

Groups of animals (n=10/group) were randomly assigned to receive the individual treatment(s) for 120 days. Doses (0.2 mL) were administered subcutaneously in a vehicle of DMSO and PEG300. Ovariectomy was performed 90 days prior to the initiation of treatment.

side of the femur facing downward in an Instron Mechanical Testing Machine (Instron 4465 retrofitted to 5500)(Canton, MA). The length (L) between the lower supports was set to 14 mm. The upper loading device was aligned to the center of the femoral shaft. The load was applied at a constant displacement rate of 6 mm/min until the femur broke. The mechanical testing machine directly measured the maximum load (F_u) (unit:N), stiffness (S) (units:N/mm), and energy absorbed (W) (unit:mJ). The axial area moment of inertia (I) (unit:mm^4) was calculated by the software during the pQCT analysis of the femoral mid-shaft. Stress (σ) (units:N/mm^2), elastic modulus (E) (unit:Mpa), and toughness (T) (units:mJ/m^3) were calculated by the following formulas: stress: $\sigma = (F_u{*}L{*}(a/2))/(4{*}I)$; elastic modulus: $E = S{*}L^3/(48{*}I)$; and toughness: $T = 3{*}W{*}(APD/2)^2/(L{*}I)$. The parameters determined to be the most sensitive to estrogen withdrawal are reported herein.

Statistical analysis was performed by Fisher's Protected Least Significant Difference test for multiple comparisons. P-values of less than 0.05 were considered as statistically significant differences.

RESULTS

The results for whole-body BMD are presented in Fig. 1. The vehicle-treated OVX control group had a lower whole-body BMD (0.197 g/cm^2) than the intact control group (0.212 g/cm^2), indicative of the significant bone loss that occurs upon ovariectomy. BMD in the 0.3, 0.5, 0.75, 1.0, and 3.0 mg/day S-4 dose groups increased to 0.204, 0.209, 0.206, 0.205, 0.205, and 0.206 g/cm^2, respectively. DHT (1 mg/day) was unable to restore the BMD in OVX animals during the treatment period. Neither DHT nor S-4 increased BMD in intact animals. However, DHT-treated animals trended lower (0.205 g/cm^2) while S-4-treated animals trended higher (0.214 g/cm^2) than intact controls. Animals receiving co-administration of bicalutamide with S-4 (0.204 g/cm^2) were not different from animals receiving S-4 alone (0.209 g/cm^2). We did not observe dose-dependent effects on whole-body BMD in this model.

Body weight and body composition data were determined using DEXA (Fig. 2). We observed a dose-dependent increase in body weight in OVX animals receiving S-4. Body weight in OVX groups treated with S-4 averaged 350 and 381 g following doses of 0.1 and 3.0 mg/day, respectively. Intact control animals had an average body weight of 308 g, and OVX resulted in an increase in body weight to 336 g. DHT treatment in intact animals resulted in an increase in body weight to 357 g. Body weight of intact animals treated with S-4 (312 g) was not different from intact controls. Body weight in animals treated with bicalutamide and S-4 averaged 347 g, and was not significantly different from that observed in OVX controls. Fat mass in the OVX control group was 41% as compared to 29% in the intact control group, illustrating the profound effect of estrogen deprivation on body composition. S-4 treatment resulted in a 2% decrease in fat mass, while DHT caused an 8% increase in fat mass in intact animals compared to intact controls. Fat mass was maximally depressed to 34% following the 0.75 mg/day S-4 dose group. Significant decreases were also observed in the 0.5 and 3.0 mg/day S-4 dose groups resulting in a fat mass of 35 and 36%, respectively. Although not all differences reached significance, S-4 treatment in all dose groups resulted in lower fat mass than the OVX control group. Co-administration of bicalutamide with S-4 partially abrogated the positive effects on fat mass seen with S-4

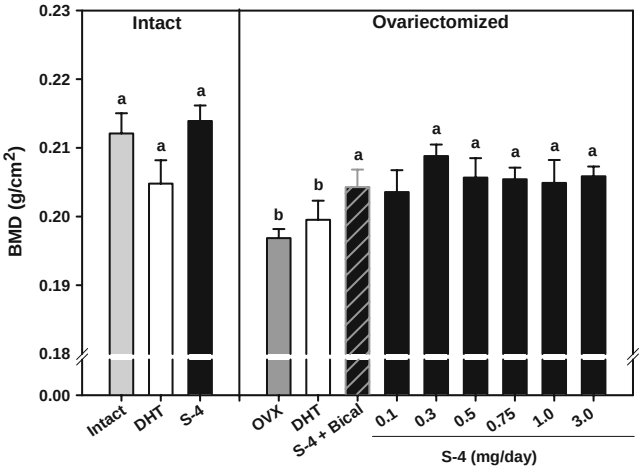

Fig. 1. Whole-body bone mineral density as measured by DEXA. Data presented as mean ± S.E.M. [a] denotes $P<0.05$ *versus* OVX controls. [b] denotes $P<0.05$ *versus* intact controls.

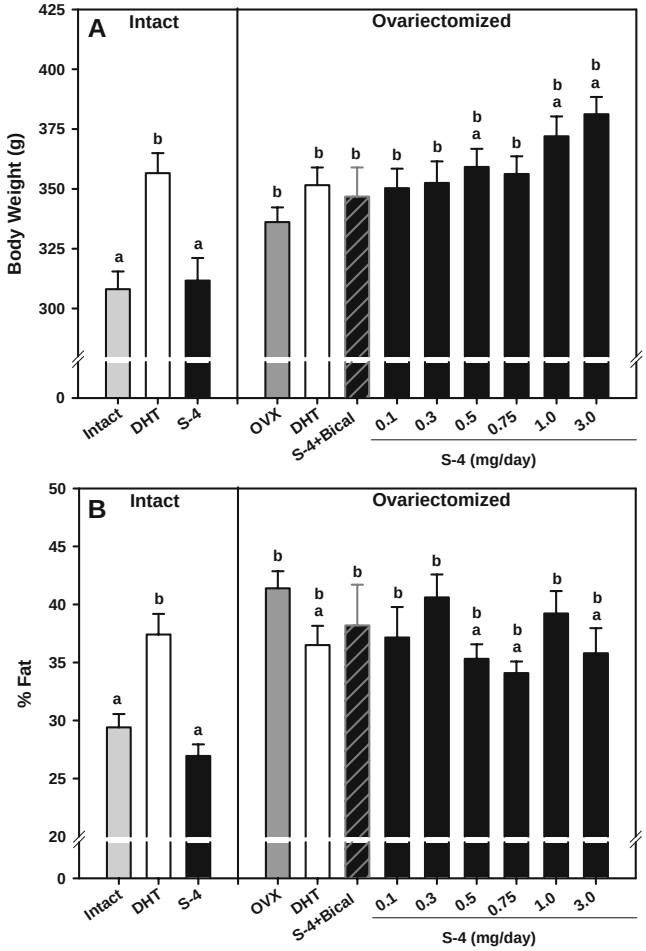

Fig. 2. Body weight and body composition as measured by DEXA. (**A**) Body weight (**B**) Percent fat mass. Data presented as mean ± S.E.M. [a] denotes $P<0.05$ *versus* OVX controls. [b] denotes $P<0.05$ *versus* intact controls.

treatment alone. DHT treatment in OVX animals resulted in a 4% decrease in fat mass.

Regional DEXA analysis was performed on the excised L5-L6 vertebrae and femur (Fig. 3). Ovariectomy negatively affected the BMD in the L5-L6 vertebra, causing a decrease from 0.234 g/cm^2 in intact animals to 0.192 g/cm^2 in OVX controls. The 3.0 mg/day S-4 dose completely restored the L5-L6 BMD in OVX animals, and significant increases in L5-L6 BMD were observed in the 0.3 mg/day dose group. Although we observed positive trends in L5-L6 BMD following S-4 treatment at all other dose levels, these differences did not reach statistical significance. DHT treatment in intact animals resulted in a significant decrease in BMD to a level not different from OVX controls, while S-4 maintained L5-L6 BMD in intact animals. DHT treatment in OVX animals partially restored the L5-L6 BMD. L5-L6 BMD in animals treated with S-4 + bicalutamide (i.e., Group 8) was not significantly different from that observed in animals treated with S-4 alone (i.e., Group 3). Cortical BMD of the femur ranged from 0.197 to 0.212 g/cm^2 in OVX and intact controls, respectively. No differences were noted in intact animals receiving S-4 or DHT. S-4 treatment resulted in higher BMD in all treatment groups as compared to the OVX control. Significant increases were observed in the 0.1, 0.75, and 3.0 mg/day dose groups, returning these animals back to the level of intact controls. Co-administration of bicalutamide with S-4 resulted in a decrease in BMD. OVX animals treated with DHT showed a slight increase in BMD.

Excised femurs were analyzed by pQCT at the mid-shaft for cortical thickness (CT), periosteal circumference (PC), and cortical content (CC), and at the distal femur for trabecular BMD (Fig. 4). Although CT decreased from 0.72 mm in intact animals to 0.66 mm following OVX, we failed to observe significant differences in CT between drug-treated groups and the OVX control group. However, we observed significant differences in PC after drug therapy. The PC was 11.98 mm and 11.45 mm in intact and OVX animals, respectively. DHT treatment resulted in a slight increase to 11.84 mm (not significant), while S-4-treated animals showed significant increases to 12.06 and 12.21 mm following 1 and 3 mg/day doses, respectively. Additionally, we observed significant differences in CC after drug therapy. CC in intact control animals was 10.3 mg/mm. OVX resulted in a loss of CC to 8.9 mg/mm. The 1.0 mg/day dose of S-4 partially restored the CC to 9.6 mg/mm, and the 3.0 mg/day dose of S-4 fully restored the CC to 10.1 mg/mm. DHT fully restored the CC to 9.9 mg/mm. Trabecular bone loss was evident in the distal femur following OVX. We observed positive trends in trabecular bone density following S-4 treatment. However, neither DHT nor S-4 fully restored trabecular BMD.

Biomechanical strength of the femur was determined by three-point bending (Fig. 5). Ovariectomy caused a reduction in the maximum load required to break the femur from 233 N in intact controls to 191 N in vehicle-treated OVX controls. S-4 treatment resulted in an increase in maximum load for both the 1.0 and 3.0 mg/day dose groups restoring them to 217 and 215 N, respectively. These values were not significantly different from that of intact controls. DHT treatment increased the femoral maximum load to 214 N.

DISCUSSION

We previously showed that S-4 maintains BMD in a model of accelerated bone loss (i.e., animals treated immediately following OVX) (6). It is well-documented that rats lose a significant amount of BMD following ovariectomy. In aged female rats, there is a rapid decline of BMD for a period of about 90 days, whereupon bone turnover stabilizes (9). The current study was undertaken to examine the effects of SARM treatment in an animal model of established osteopenia. In order to evaluate the treatment potential of S-4, we withheld drug therapy for 90 days following OVX to allow bone loss to occur and then treated the animals for 120 days with the compounds of interest. This osteopenic model represents a treatment paradigm for an established disease, whereas the previous study was focused on preventing bone loss resulting from hormone deficiency. Although osteoporosis awareness is considerably more prevalent today than even just a few years ago, many patients are not diagnosed until after a considerable amount of bone loss has already occurred. Therefore, there is an overwhelming need for an agent that could rebuild lost bone. We hypothesized that a nonsteroidal SARM would stimulate bone apposition in the skeleton of osteopenic rats.

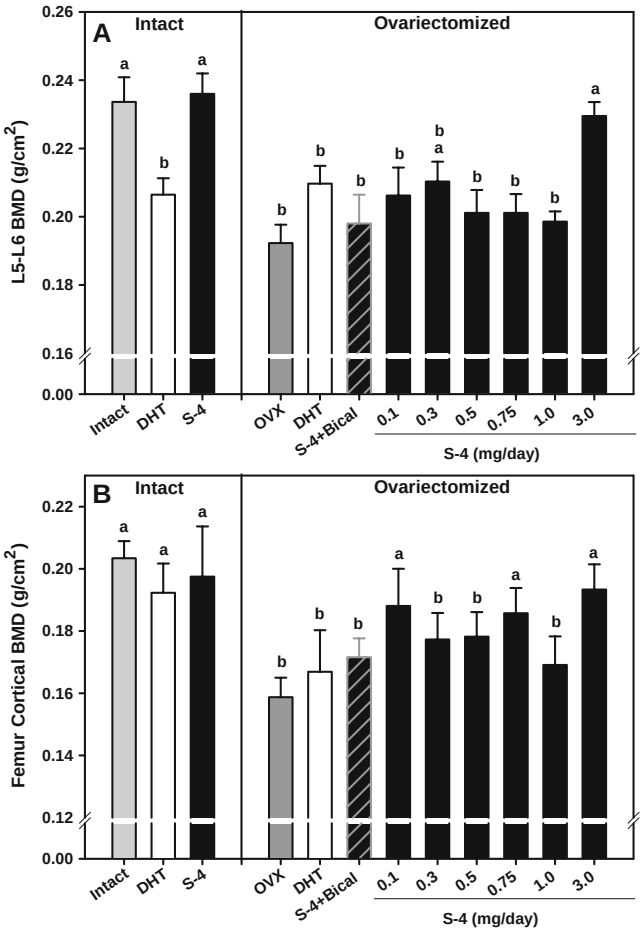

Fig. 3. Bone mineral density as measured by DEXA in excised bones. (**A**) Lumbar vertebrae (L5-L6). (**B**) Femur cortical BMD. Data presented as mean ± S.E.M. [a] denotes $P<0.05$ *versus* OVX controls. [b] denotes $P<0.05$ *versus* intact controls.

The vast majority of current treatments for osteoporosis act through an anti-resorptive mechanism. Thus, increases in BMD are due to prolongation of mineralization and reduction of remodeling space (1). Anabolic mediators of bone formation would act through a completely different mechanism, and have the potential to increase BMD to a greater degree than agents that only inhibit bone resorption (1). In this study, female rats with established bone loss were used to evaluate the therapeutic potential of S-4 in osteopenia. The results of this study demonstrate that S-4 exerts positive effects on the skeleton of osteopenic rats. Whole-body and regional BMD as measured by DEXA, as well as biomechanical indices of bone quality, clearly showed that S-4 improves bone quality and quantity in rats. Whole-body BMD was fully restored in animals receiving >0.1 mg/day S-4. Regional analysis of the L5-L6 and the femur by DEXA showed that S-4 completely restored BMD. Higher resolution analysis by pQCT provided more stringent confirmation of the positive skeletal effects of S-4 on cortical bone in the femur. Mechanical strength-testing demonstrated that the changes observed by DEXA and pQCT were physiologically relevant, as the breaking strength of the femur was significantly improved compared to OVX controls and not statistically different from the level of intact controls, indicative of the anabolic effects of S-4 on cortical bone.

In the parameters measured by DEXA, we observed variability between similar dose groups and the lack of a clear dose response in some parameters. This variability could be a result of all doses producing maximal effect in the measured parameter, pharmacokinetic variability, or DEXA precision. Since the Lunar Prodigy™ is a clinical DEXA optimized for scanning human patients, some sensitivity is lost when measuring animals with body weights in the 300–400 g range. Although, we did not observe a clear dose response in the parameters measured by DEXA, the bone parameters in drug-treated animals were consistently improved compared to vehicle controls. However, we did not measure S-4 plasma concentrations or perform more stringent analyses on the excised bones of each group. Therefore, we are unable to speculate as to the contributions of the factors listed above. Previous studies in our laboratory to examine the protective skeletal effects of S-4 in an acute post-OVX model of postmenopausal bone loss showed that S-4 prevented bone loss following ovariectomy. Since bone loss prevention could result from either increased bone formation or decreased bone resorption, we could only hypothesize as to the primary mechanism by which S-4 prevented bone loss. However, S-4 only partially maintained the trabecular density of the distal femur in our previous study (6). Known anti-resorptive agents, such as estrogen and bisphosphonates, fully maintain trabecular density in models of accelerated bone loss. As a whole, these data suggest that S-4 likely operates through two mechanisms. S-4 fully stimulates bone formation and exhibits weaker antiresorptive activity, suggesting that SARMs provide a unique mechanistic approach to osteoporosis that may be additive or even synergistic with existing anti-resorptive therapies.

In an effort to reduce the number of animals required for the current study, as well as the previous study (which were run in parallel), the OVX control group was used for comparison in both studies. For this reason, the excised bone parameters (pQCT and mechanical strength analysis) for the OVX control group were performed on samples which were 120 days post ovariectomy as opposed to 90 days post ovariectomy, which would be a true baseline measurement. Since bone turnover stabilizes around day 90 in OVX rats (9), these samples provided good approximations of baseline measurements for the excised bone parameters. The DEXA analysis of whole-body BMD, body weight, and percent fat mass were performed in anesthetized animals on day 90 for the OVX control group.

Although S-4 treatment resulted in an increased body weight, these animals exhibited a lower fat mass than the OVX control animals. These results were similar to those observed in our previous study (6). As discussed in our previous study, increases in lean mass are beneficial to the skeleton in two ways. First, lean mass may indirectly increase BMD due to increasing skeletal stresses, which stimulate bone formation (10). Second, increased muscle mass and strength may reduce the risk of falling and thereby reduce the risk of fracture (11). Therefore, S-4 may be more advantageous for clinical use than a purely bone anabolic agent. Interestingly, DHT treatment in intact animals resulted in an 8% increase in fat mass compared to intact controls. This is likely due to the DHT-induced inhibition of luteinizing

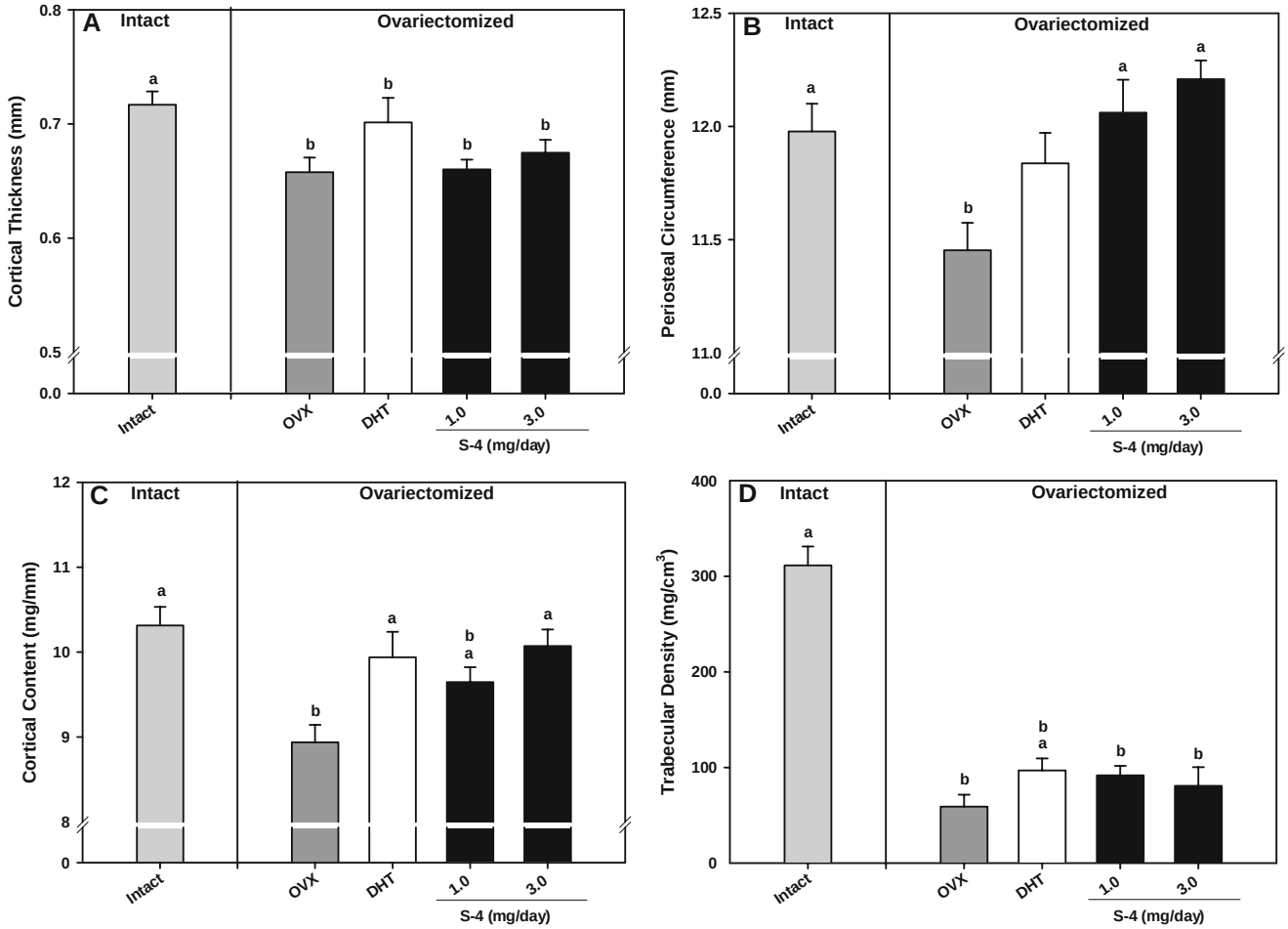

Fig. 4. pQCT analysis of the mid-shaft and distal femur. (**A**) Cortical thickness at the femoral mid-shaft. (**B**) Periosteal circumference at the femoral mid-shaft. (**C**) Cortical content at the femoral mid-shaft. (**D**) Trabecular density of the distal femur. Data presented as mean ± S.E.M. [a] denotes $P<0.05$ *versus* OVX controls. [b] denotes $P<0.05$ *versus* intact controls.

hormone and follicle-stimulating hormone release from the pituitary resulting in a decrease in estrogen levels.

In an effort to delineate androgen effects in cortical *versus* cancellous bone, we analyzed the mid-shaft and distal femur by pQCT. Although we did not observe differences in cortical thickness between the OVX groups, the drug-treated groups exhibited increased periosteal circumference (PC), cortical content (CC), and biomechanical strength. S-4 fully restored CC at the 3 mg/day dose level and partially restored CC at the 1 mg/day dose level. However, PC and biomechanical strength were fully restored by both 1 and 3 mg/day doses. These data show that S-4 and DHT exert similar effects in cortical bone. The lesser effects in trabecular bone were likely due to the loss of pre-existing architecture on which to rebuild lost bone. In cortical bone, our results are in agreement with those reported by Hanada *et al.*, which showed increases in cortical BMD with androgens (3). Additionally, we have shown that the androgenic effect in cortical bone translates directly to biomechanical strength (Fig. 5). In trabecular bone, we observed positive trends in S-4-treated groups. Although the differences did not reach significance, S-4-treated animals had a higher average trabecular BMD. Our DHT control group showed significant differences from OVX controls. Studies by Tobias *et al.* found that DHT was able to partially restore cancellous bone in OVX rats (5). They noted that DHT primarily increased trabecular thickness as opposed to trabecular number at the distal femur. Since the trabecular effects of androgens are due to

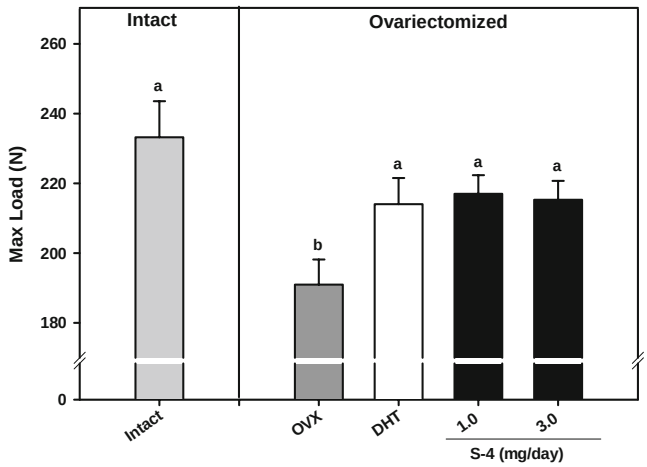

Fig. 5. Femoral maximum load determined by 3-point bending. Data presented as mean ± S.E.M. [a] denotes $P<0.05$ *versus* OVX controls. [b] denotes $P<0.05$ *versus* intact controls.

increasing trabecular thickness instead of stimulating the formation of new trabeculae, we did not expect androgens to fully restore trabecular BMD.

Parathyroid hormone has been shown to fully restore both cancellous and cortical bone in ovariectomized rats (12–14). In some cases, parathyroid hormone increased BMD in OVX animals to a level greater than that observed in sham-operated intact controls (12–14). Although the efficacy of S-4 is less than that reported for parathyroid hormone in bone, S-4 fully restored whole-body BMD, L5-L6 BMD, femur cortical BMD, CC, and femur biomechanical strength. However, direct comparison between parathyroid hormone and S-4 would be necessary to confirm these differences. Importantly, S-4 and other SARMs are amenable to oral administration, identifying them as the only medical possibility for bone restoration without the need for parenteral delivery.

CONCLUSION

As a whole, these data suggest that S-4 is likely to be efficacious in reducing the fracture risk in patients with osteoporosis through direct stimulation of bone and muscle (7). Previous studies in our laboratory demonstrated that unlike DHT, S-4 acts as a partial androgen receptor (AR) agonist in the prostate while exhibiting full AR agonism in muscle (i.e., prostate weights in castrated male rats treated with 1 mg/day DHT or S-4 were 192% and 35% of intact vehicle treated controls, respectively). In the current study, S-4 performed as well or better than DHT across all parameters examined, indicating that S-4 is a full AR agonist in bone. From previous studies, we have shown that S-4 is orally bioavailable (8), tissue-selective (7), and does not cross-react with other steroid hormone receptors (15). The current study demonstrates that S-4 increases bone apposition in osteopenic rats. It is possible that SARM treatment will result in greater fracture prevention than would be predicted by the preclinical effect in bone due to a synergistic effect on the musculoskeletal and skeletal systems. S-4 and other SARMS exert direct skeletal effects through bone anabolism and the prevention of bone resorption. These actions result in improved bone quality and strength. Furthermore, SARMs increase skeletal muscle mass and strength, likely leading to increased stability when walking (i.e., reduction in falls) and increased mobility (i.e., bone mechanical loading). SARMs provide a combination therapy approach (i.e., muscle and bone) to the treatment of osteoporosis through a single therapeutic agent. The combination of attributes possessed by S-4 may provide a unique approach to the treatment and/or prevention of osteoporosis. Historically, the OVX female rat model has robustly predicted clinical efficacy in post-menopausal osteoporosis; however, future studies in higher species with bone remodeling more similar to humans would be needed to further validate these data.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Juhyun Kim, Dr. Jun Yang, Dr. Victor Shen, and Dr. Mitch Steiner for their technical assistance and advice.

REFERENCES

1. Rubinand MR, Bilezikian JP. New anabolic therapies in osteoporosis. Endocrinol Metab Clin North Am. 2003;32:285–307.
2. Vanderschuerenand D, Vandenput L. Androgens and osteoporosis. Andrologia. 2000;32:125–30.
3. Hanada K, Furuya K, Yamamoto N, Nejishima H, Ichikawa K, Nakamura T, *et al.* Bone anabolic effects of S-40503, a novel nonsteroidal selective androgen receptor modulator (SARM), in rat models of osteoporosis. Biol Pharm Bull. 2003;26:1563–9.
4. Leaand CK, Flanagan AM. Physiological plasma levels of androgens reduce bone loss in the ovariectomized rat. Am J Physiol. 1998;274:E328–35.
5. Tobias JH, Gallagher A, Chambers TJ. 5 alpha-Dihydrotestosterone partially restores cancellous bone volume in osteopenic ovariectomized rats. Am J Physiol. 1994;267:E853–9.
6. Kearbey JD, Gao W, Narayanan R, Fisher SJ, Wu D, Miller DD, *et al.* Selective Androgen Receptor Modulator (SARM) treatment prevents bone loss and reduces body fat in ovariectomized rats. Pharm Res. 2007;24:328–35.
7. Yin D, Gao W, Kearbey JD, Xu H, Chung K, He Y, *et al.* Pharmacodynamics of selective androgen receptor modulators. J Pharmacol Exp Ther. 2003;304:1334–40.
8. Kearbey JD, Wu D, Gao W, Miller DD, Dalton JT. Pharmacokinetics of S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro- 3-trifluoromethyl-phenyl)-propionamide in rats, a non-steroidal selective androgen receptor modulator. Xenobiotica. 2004;34:273–80.
9. Wronski TJ, Cintron M, Dann LM. Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. Calcif Tissue Int. 1988;43:179–83.
10. Ott S. Osteoporosis and Bone Physiology. 1998.
11. Lipsitz LA, Nakajima I, Gagnon M, Hirayama T, Connelly CM, Izumo H, *et al.* Muscle strength and fall rates among residents of Japanese and American nursing homes: an International Cross-Cultural Study. J Am Geriatr Soc. 1994;42:953–9.
12. Iwaniec UT, Samnegard E, Cullen DM, Kimmel DB. Maintenance of cancellous bone in ovariectomized, human parathyroid hormone [hPTH(1–84)]-treated rats by estrogen, risedronate, or reduced hPTH. Bone. 2001;29:352–60.
13. Samnegard E, Akhter MP, Recker RR. Maintenance of vertebral body bone mass and strength created by human parathyroid hormone treatment in ovariectomized rats. Bone. 2001;28:414–22.
14. Samnegard E, Iwaniec UT, Cullen DM, Kimmel DB, Recker RR. Maintenance of cortical bone in human parathyroid hormone(1–84)-treated ovariectomized rats. Bone. 2001;28:251–60.
15. Yin D, He Y, Perera MA, Hong SS, Marhefka C, Stourman N, *et al.* Key structural features of nonsteroidal ligands for binding and activation of the androgen receptor. Mol Pharmacol. 2003;63:211–23.