**INTRODUCTION**

Pediatric disuse osteopenia is often found in children with spastic quadriplegic cerebral palsy or spina bifida who bear minimal weight. Causes for osteopenia are multifactorial, but disuse and immobilization are important contributing factors which result in significant fracture risk in immobilized limbs. Creating models for disuse osteoporosis in a growing, pediatric population, as well as designing interventions to prevent fracture incidence is of particular interest.

Rodent hindlimb suspension (HLS) has been well characterized as a model for disuse osteopenia compared to ground controls (GRND), but the skeletal effects have not been fully described in mice undergoing rapid growth. The purpose for this experiment is to investigate the effects of disuse HLS in a rapidly growing mouse model, and to determine the effectiveness of alendronate (ALN) treatment on the prevention of pediatric disuse-associated bone loss.

**METHODS**

21 day male Balb/c mice were acclimatized for 1 week prior to experiment. Mice were grouped according to environment (HLS vs. GRND), treatment (ALN 0.219 mg/kg/week vs. untreated) and experiment duration (3 wk vs. 7 wk) with 8-11 mice per group. An additional 3 mice were euthanized at the start of the experiment as baseline controls (BASE). Prior to euthanasia, blood was drawn by intracardiac puncture, and serum was analyzed for osteocalcin and TRACP5b activity. Tibiae and femora were dissected, imaged by μCT, and mechanically tested to failure (tibiae in torsion, femora in 4-point bending).

Age-related changes between 3 and 7 weeks within common environment and treatment groups were assessed by t-test, as were ALN effects in age-matched groups. To determine HLS vs. GRND effects independent of body weight, parameters were assessed by ANCOVA with environment as the factor and body weight as the covariate.

**RESULTS**

As expected, untreated control mice gained significant body mass over the course of the experiment (Fig 1). Body mass changes were reflected in μCT, 4-point bending, and torsion parameters, with continued growth in size, strength, and stiffness of tibiae and femora from 3 to 7 weeks (Table 2,3 GRND-UNT 3 vs. 7 Wk +). Trabecular structural properties remained near baseline levels in the femur and reduced slightly by 7 weeks in the tibia (Table 4). Reductions in TRACP5b and OCN serum markers suggest an age-related decline in cellular activity between 3-7 weeks in GRND UNTs (Table 1).

HLS resulted in significant reductions in body weight vs. GRND (Fig 1). However, body weight and cortical parameters continue to increase through 7 weeks of HLS, indicating continued growth, but at a reduced rate vs. GRND (Fig 2,3 HLS-UNT 3 vs. 7 Wk +). HLS induced significant decrements in tibial cortical μCT parameters at 3 and 7 weeks compared to GRND, and these changes were reflected in decreased tibial stiffness at 7 weeks and torque at 3 and 7 weeks (Table 2 HLS vs. GRND UNT*). Reductions in femoral cortical thickness and bending moments were seen at 3 weeks, but these changes were not enough to be reflected in femoral 4-point bending parameters (Table 3). Trabecular parameters were significantly reduced in tibia and femur at 3 and 7 weeks following HLS compared to GRND controls, demonstrating additional loss below baseline values (Table 4 HLS vs. GRND UNT*). Serum OCN was reduced in HLS mice at 3 weeks, while serum TRACP5b was elevated compared to matched controls, and a later phase at 7 weeks where bone resorption becomes upregulated due to continued disuse.

ALN was unable to alleviate HLS-induced cortical bone loss in the tibia, but it improved cortical mass and mechanical parameters in the femur, suggesting site-specific sensitivity to the bisphosphonate treatment. Increased trabecular bone mass was evident in both bones when both HLS and GRND mice were treated with ALN.

**DISCUSSION**

Pediatric disuse osteopenia combines bone wasting effects of disuse with bone growth due to skeletal maturation. Tibial cortical bone was more sensitive to HLS-induced bone loss than femoral bone when controlling for weight loss effects. However, trabecular compartments from both bones were equally affected by disuse. Serum measures suggest that bone loss occurs in two phases- an early phase at 3 weeks, where bone formation is reduced compared to age-matched controls, and a later phase at 7 weeks where bone resorption becomes upregulated due to continued disuse.

ALN was unable to alleviate HLS-induced cortical bone loss in the tibia, but it improved cortical mass and mechanical parameters in the femur, suggesting site-specific sensitivity to the bisphosphonate treatment. Increased trabecular bone mass was evident in both bones when both HLS and GRND mice were treated with ALN.

These findings suggest that HLS during periods of rapid growth may be a useful model for studying the effects of anti-resorptives on disuse-associated bone loss in a pediatric population.

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**REFERENCES**