Introduction
Attention Deficit Hyperactivity Disorder (ADHD) is a common childhood disorder, with a prevalence of six to nine percent in the U.S. (Dopheide and Pliszka 2009). It is most often treated with psychostimulants of which methylphenidate (MP), sold as Ritalin, accounts for upwards of 90 percent of prescriptions (Goldman et al. 1998). The use of stimulants to treat ADHD has doubled in the last decade, with approximately five percent of all U.S. children and adolescents now taking them.

Although MP is generally well tolerated, numerous clinical studies have suggested that MP adversely affects growth (Faraone et al. 2008). This effect has been discounted by some as not being clinically significant (Roche et al. 1979; NIH Consensus Committee 1998), while others have reported an attenuation of negative growth effects upon cessation of treatment (Pliszka et al. 2006). However, since increasing numbers of adolescents are being treated with MP, thorough investigation of its potential negative skeletal effects is warranted.

To date, only limited animal studies on the skeletal effects of MP have been reported, and none have evaluated its effects on biomechanical integrity. This study was designed to evaluate the hypothesis that long-term MP treatment is detrimental to adolescent rat skeletal development and to characterize the skeletal responses of adolescent rats to chronic MP treatment at two clinically relevant dosages.

Methods
Four-week old male Sprague-Dawley rats were randomized into three groups: low-dose (MP low; n=12), high-dose (MP high; n=8) and vehicle (Veh; n=8). MP was administered daily via drinking water over a 2.5 month period. Veh rats received water with no drug, while the MP high and MP low rats received 30 or 5mg/kg MP, respectively, for one hour followed by 15 or 2mg/kg, respectively, for another seven hours.

These dosages resulted in plasma MP levels corresponding to low and high clinical doses (Kuczenski and Segal 2002). Rats were weighed daily and the doses were adjusted accordingly. At study completion, the rats were sacrificed and their left femurs were harvested and evaluated by dual energy x-ray absorptiometry (DXA) using a Lunar PIXImus II (GE-Lunar) to determine bone mineral density (BMD), bone mineral content (BMC) and area. Length, anterior-posterior (AP) and medial-lateral (ML) diameters were then measured using digital calipers.

Finally, energy to failure, stiffness and ultimate force were calculated subsequent to three-point bending tests using an Electroforce 3200 (Bose). In addition, left tibias were harvested for histologic growth plate analyses. Tibias were fixed in ten percent formalin and processed for paraffin embedding and sectioning. Masson’s Trichrome stain was employed and average growth plate thickness was measured from photomicrographs. One-way ANOVA tests were performed on each outcome with p-values less than 0.05 considered significant. When significance was found, Dunnett’s pairwise comparisons were performed to identify the source of the differences.

Results
Effects of MP on weight were significant, yet contrasting. Compared to Veh, MP high rats gained 12 percent less. In contrast, MP low rats gained 23 percent more weight than the Veh rats over the study period. Of the measured skeletal parameters, only BMC was significantly different with a nine percent reduction seen in MP high rats as compared to Veh. However, strong trends were evident in all other parameters. There was a dose dependent decrease in mineralization, evident in both BMD, which was unaffected in MP low rats, but measured five percent lower in MP high rats, and BMC, which was four percent lower in MP low rats.

Femoral size also showed dose dependent reductions, with MP low resulting in reductions of three percent, one percent and one percent for area, length and AP diameter, respectively, and MP high leading to reductions of four percent, two percent and four percent for area, length and AP diameter, respectively. ML diameter was reduced by three percent in both MP high and low groups.

Mechanical properties also revealed dose dependent decreases. Energy to failure decreased by nine percent in MP low and 21 percent in MP high rats, stiffness was
reduced by seven percent in MP low and 11 percent in MP high, and ultimate force was decreased by eight percent in MP high. Similar to the body weight data, growth plate measurements revealed contrasting trends for MP with growth plates 12 percent thinner than Veh in the MP high group and 28 percent thicker in the MP low group.

Discussion
This study examined the effects of chronic MP treatment on growth and the structural integrity of adolescent rat bone. High dose MP consistently resulted in decreased values for all properties examined, but the only statistically significant effects of MP detected were for body weight and BMC. The decreases in weight and growth plate thickness in MP high rats indicate that at this dose, these animals do experience a growth deficit, as previously reported in humans (Swanson et al. 2007). At low doses, other studies have also shown a possible weight increase (Beckman et al. 2008), suggesting there may be a threshold dose below which growth deficits are not evident. Weight and growth plate data coupled with indications of dose dependent decreases in BMC, length, energy to failure and stiffness suggest a complex relationship between chronic MP administration and negative skeletal effects.

Limitations to this study include confounding weight data, a small sample size and the use of an animal model. High dose animals were significantly smaller as measured by weight, but there is no statistical evidence to suggest that their femora were in fact smaller (growth plate, length, diameter), although data trends suggest this possibility. This indicates that the significant decrease seen for BMC may be independent of reduced bone size and therefore may have significant clinical ramifications.

All of the outcomes measured in this study were characterized by high variability. Accordingly, this study may not have been sufficiently powered to identify all statistically significant results and further studies will be required to ascertain whether or not the identified trends reflect significant differences between groups. Lastly, because this study was performed in an animal model, clinical studies will be needed to confirm the relevance of these findings to humans.

Although this study is by no means conclusive, the data do support clinical evidence of growth deficits in children and adolescents treated with MP, particularly at high doses. Furthermore, they suggest that chronic MP treatment may also result in weaker, less mineralized bone. This has immediate implications for active adolescents who may be at an increased risk for fracture, as well as future implications for these patients if MP treatment results in reduced peak bone mass, potentially increasing their susceptibility to osteoporosis.

Additional animal studies, as well as clinical studies, designed to elucidate the molecular mechanisms behind these bone-related changes would be of tremendous benefit to the medical community and provide guidance that will enable better informed dosage decisions in order to reduce the potential adverse skeletal effects of chronic MP treatment.

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