INTRODUCTION:
It is thought that progression of scoliosis occurs at least in part as a result of asymmetrical forces on the vertebrae creating differential growth and hence wedging deformity. The response of bone growth to loading, and especially to diurnal variations in loading, is not known quantitatively. Longitudinal growth of long bones and vertebral occurs in growth plates, where new cells produced in the proliferative zone enlarge and synthesize extracellular matrix in the hypertrophic zone, and the tissue thus formed becomes ossified. Growth can be considered as the product of the levels of cellular activity (proliferation and hypertrophy) in these two zones.

The aim of this study was to determine whether the amount of growth response to mechanical compression differed with night-time or day-time loading, relative to full time loading. We hypothesized that the growth modulation effect of half-time loading would be half that of full time loading, irrespective of whether the half-time loading was imposed during the day or night. Also, we investigated the relative contributions of changes in chondrocytic proliferation and of changes in chondrocytic enlargement to the mechanically induced growth modulation. The study was performed in the tail vertebral growth plates, and in the more rapidly growing proximal tibial growth plates of young rats.

METHODS:
Mechanical compression was applied across the growth plates of the 7th caudal vertebra and of the right proximal tibia of 35-day-old Sprague-Dawley rats. The external loading apparatus used compression springs in an Ilizarov-style construct, adjusted to apply nominally 0.1 MPa stress. Two pins were used to transect respectively the 6th and 8th caudal vertebrae, the right tibial proximal epiphysis and the right tibial diaphysis. The apparatus was installed under general anesthesia (Ketamine 80 mg/kg and Xylazine 10 mg/kg) with post-operative analgesia (Buprenorphine 0.05 mg/kg).

Figure 1: Radiograph showing compression apparatus installed in the tail and right tibia of a rat.

Four groups of animals were tested: 24/24 hour (full-time loading); 12/24 hour (day-loading); 12/24 hour (night-loading); and 0/24 hour (sham instrumented). There were four or five animals per group. In half-time loaded animals, spring forces were removed or applied at 'lights-on' and 'lights-off' times in an artificial light cycle to which animals had previously become acclimated. Calcine (15 mg/kg) was administered systemically 24 hours prior to euthanasia, and BrdU (25 mg/kg) 30 minutes before death. All live animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee. After 8 days, animals were euthanized and the loaded and within-animal control growth plates were removed, fixed [1], and embedded in Epon-Araldite. One micron sections were cut and mounted, and imaged at 1300 x 1030 pixel resolution using a Zeiss microscope. Twenty-four hour growth was measured from the fluorescent calcine label. In the proliferative zone the labeling index was paradoxically increased (but not significantly). Mean growth rates were 43 microns/day (vertebral growth plates) and 237 microns/day (tibiae).

DISCUSSION AND CONCLUSIONS
Mechanical compression of 0.1 MPa suppressed growth of vertebrae and tibiae (that grow at very different rates) by a similar proportional amount relative to controls, and taking into account the sham effect evident in tibiae. The half-time loading effect on growth approximated half that of full-time loading, with no difference between night-time and day-time loading. Daily longitudinal growth is approximately equal to the product of the number of new cells produced per day and the final chondrocytic height. Therefore, the percentage change in growth was expected to be equal to the sum of the percentage changes in labeling index, number of proliferating cells, and their final height. The relative estimates of proliferating cell numbers and final cell height appeared to have this additive relationship with growth, but the labeling index was paradoxically increased (but not significantly). Mean growth rates were 43 microns/day (vertebral growth plates) and 237 microns/day (tibiae).