BONE MINERAL ACCRETION, ENDOCHONDRAL ELONGATION AND METABOLIC ACTIVITY DURING POST-NATAL SKELETAL MATURATION IN THE SPRAGUE-DAWLEY RAT

Introduction: Long term survivors of pediatric solid tumors treated with chemotherapeutic protocols have been identified as a population who are at elevated risk for osteopenia and precocious osteoporosis as young adults.(1) While the etiology for this morbidity is multifactorial, age at which therapy was begun relative to the stage of skeletal maturity has been implicated as one of several factors of this late effect (2). These factors have yet to be studied thoroughly in an animal model. The purpose of this study was to describe the temporal phases of skeletal metabolic activity, growth plate function and mineral accretion in the young, growing rat, a model which simulates the fast growth period of adolescence in humans. This data will serve as a basis for further study in this animal model of chemotherapy effects and prophylactic or post-treatment interventions on bone mineral density.

Materials and Methods: With institutional approval, 28-day old male female Sprague-Dawley rats were obtained and subjected to semi-weekly in-vivo bone densitometry, radiography, blood collections, and body weight measurement. DXA-derived femoral and lumbar bone mineral content and densities were determined using the Piximus2 densitometer (3). Tibial lengths were measured from digitized plain film radiographs (4). Serum levels of osteocalcin were measured by IRMA as an indicator of osteoblastic bone formation (5). Serum levels of tartrate-resistant acid phosphatase 5b were measured by ELISA, as an indicator of osteoclastic resorption (6). Additional male rats 6-, 10-, 13-, 16-, and 52-weeks of age were obtained for histologic studies of the proximal tibial growth plate. Oxytetracycline (50mg/kg, IP) was injected 48-hours before euthanasia to determine longitudinal growth velocity. BrdU (25 mg/kg, IP) was injected 30-minutes before euthanasia to determine the proliferative activity of growth plate chondrocytes via calculation of an immunohistochemical labeling index. The animals were killed by CO2 inhalation, and the proximal tibiae were dissected, fixed in 70% ethanol, embedded in MMA and sectioned at 5µm thickness. Total and zonal growth plate height and matrix area fraction, 48-hours after OTC histologic measurements were fitted to a Gompertzian growth curve (solid), displaying features of exponential growth (R2>0.93).(7) Body weight also followed a similar decay pattern, though the failure to reach an asymptotic plateau is likely due to extraskeletal gain rather than maturative skeletal growth. Metabolic activity in the young rat favored bone formation, with the decline in osteoblast activity corresponding to the plateau in femoral and lumbar BMD. Temporal patterns of growth were not different between sexes. Osteoclast activity remained relatively constant throughout the time observed, while osteoblast activity declined 3-fold from high levels of formation during exponential growth to a static level upon plateau through 18 weeks of age. (Fig 3).

Results: An early phase of exponential gains in body weight, tibial elongation and mineral accretion was identified through 10 weeks of age. An inflection toward less aggressive tibial elongation was seen through 16 weeks, where decay in the growth rate was evident. (Fig 1)

Discussion: The plotted curves of both femoral and lumbar BMD and tibial length over time were found to conform to the Gompertzian model of growth (R^2>0.93).(7) Body weight also followed a similar decay pattern, though the failure to reach an asymptotic plateau is likely due to extraskeletal gain rather than maturative skeletal growth. Metabolic activity in the young rat favored bone formation, with the decline in osteoblast activity corresponding to the plateau in femoral and lumbar BMD. Temporal patterns of growth were not different between sexes. The Sprague-Dawley rat accrues bone density early in life, making it a reasonable model for examination of bone mineral density effects.


Acknowledgements: David G. Murray Endowment

Figure 1: Tibial elongation. Data shows mean tibial length, ± 1SD, and is fitted to a Gompertzian growth curve (solid), displaying features of exponential gain (dashed), growth rate decay and plateau.

A similar pattern was seen for both femoral and lumbar mineral accrual, though the plateau in bone density was reached 3 weeks later than that for tibial elongation. (Fig 2)

Figure 2: Femoral bone mineral accrual. Data shows mean femoral BMD, ±1SD, fitted to a Gompertzian growth curve (solid), displaying features of exponential gain (dashed), growth rate decay and plateau.

Growth rates determined from OTC-histologic measurements were strongly correlated to the observed changes in elongation via radiographic measurement. Similarly, the vertical height of the total growth plate, and proliferative, transitional and hypertrophic zones showed strongly positive correlation (R^2>0.85) to both daily growth rate and the relevant radiographic length measurement. Matrix area fractions of these zones of the growth plate were inversely correlated to both measures of elongation (R^2>0.5), as were both the height and matrix fraction of the reserve zone (R^2>0.93). Decreased chondrocyte proliferative activity was also associated the decay of growth rate. Osteoclast activity remained relatively constant throughout the time observed, while osteoblast activity declined 3-fold from high levels of formation during exponential growth to a static level upon plateau through 18 weeks of age. (Fig 3).

Figure 3: Serum measurement of bone cell metabolic activity. Osteocalcin (dashed) was measured by IRMA to assess bone formation rate, and osteoclast resorptive activity was assessed by ELISA for active circulating TRAP-5b (solid). Data is mean value ± 1SD for n=3 animals.