INTRODUCTION:

Suboptimal skeletal development may affect long-term bone strength and increase the incidence of fracture during growth and at maturity. Estrogen and other sex steroids contribute to sexual dimorphism in bone architecture and thus bone strength. Increased estrogen levels in females at puberty inhibit periosteal modeling resulting in smaller bones compared to males. As a result a delay in puberty should result in an increased periosteal diameter potentially producing stronger bones because the resistance to bending or torsional forces is exponentially related to bone diameter. However, an animal model of delayed puberty (suppressed estrogen levels) has reported short-term decreases in peak moment and stiffness without changes in total area or bone area (1). In clinical studies the delay of menarche and infrequent menstrual cycles decrease estrogen levels during adolescence and subsequent bone mass accrual (2). Furthermore, stress fractures were reported to have a higher correlation to the age of menarche than bone mineral density (3). Recruitment and function of osteoblasts and osteoclasts at the periosteal and endosteal surfaces determine bone dimensions but have not been thoroughly investigated during pre-pubertal growth. Therefore the purpose of this study was to determine the recruitment and function of bone formation on the periosteal and endosteal surfaces and the resultant bone strength in female rats with delayed puberty.

METHODS:

At 23 days of age, female Sprague Dawley rats (Charles River) were randomly assigned into a control group (C) (n=15) and three experimental groups that received injections of Gonadotropin releasing hormone antagonists (GnRH-a) (Zentaris GmbH) intraperitoneally (0.2ml) for a 29 day period. The experimental groups were separated into a group that injected 125 ug/dose daily (D1) (n=15), 250 ug/dose daily (D2) (n=15) and 500 ug/dose, 5 days per week (D3) (n=15). All animals were monitored daily for vaginal opening which is an indicator of the onset of puberty. Rats were given two calcein injections (10mg/kg), 10 days and 2 days prior to sacrifice at 50 days of age. Uterine weights were measured. Left femora were mechanically tested into a group that injected 125 ug/dose daily (D1) (n=15), 250 ug/dose daily (D2) (n=15) and 500 ug/dose, 5 days per week (D3) (n=15). All animals were monitored daily for vaginal opening which is an indicator of the onset of puberty. Differences were detected using a One-way ANOVA (p < 0.05). The study was approved by the Institutional Animal Care and Use Committee at Brooklyn College (City University of New York).

RESULTS:

A delay in the timing of puberty through GnRH-a injections was confirmed by the delay in the day of vaginal opening of the experimental groups compared to control. 50% of the animals in D1 did not reach puberty prior to sacrifice. 93% of the animals in D2 and D3 groups did not reach puberty. The significant decrease in uterine weight by 75.5% was a further indicator of the efficacy of the protocol to delay pubertal development. Diaphyseal strength (peak moment) was lower in the GnRH-a groups (Table 1). However, total cross-sectional area (T.Ar) and cortical bone area (Ct.Ar) did not change in the experimental groups. Modeling results in large size and architecture changes in growing bone. In the current study, the bones increased in total area while drifting in the posterior-lateral direction. On the endocortical surface, formation occurred on surface #1 while resorption occurred on surface #2 (Figure 1). A periosteal expansion on surface #3 increased the overall size of the bone. Periosteal labeled surface (LS/BS) was significantly increased in the GnRH-a groups (D2, D3) compared to control (Table 1). The majority of this formation surface was single labeled. Endocortical labeled surface (LS/BS) decreased as the GnRH-a dosage was increased, but the result was not statistically significant (Table 1). The percentage of labeled surface from double labels decreased from 98.5% in the control group to 76% in the GnRH-a group.

DISCUSSION:

GnRH-a injections administered to pre-pubescent rats delayed the onset of puberty for the duration of the injection period. GnRH-a injections diminished the strength of the femoral diaphysis, however the decrease could not be attributed to a significant cross-sectional area change. Total cross-sectional area and cortical bone area were similar between groups. However, endosteal and periosteal surface activity differed between groups. Animals with a delayed puberty maintained bone area through different mechanisms compared to control animals. The periosteal osteoblast recruitment increased in the GnRH-a groups in a possible attempt to compensate for diminished mechanical strength. Therefore, decreased mechanical strength may be due to either inferior material properties, increased periosteal resorption on the anterior-medial surface or decreased cortical width due to the trend towards decreased endosteal labeled surface. Endosteal apposition occurs primarily during adolescent growth (4); therefore suppression of endosteal formation activity during delayed puberty may have a long-term effect on cortical thickness and mechanical strength.

REFERENCES:


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