INTRODUCTION

Optimal bone strength is imperative in order to avoid fracture later in life. Multiple factors affect the structural development of the skeleton; in particular estrogen levels during growth are an important factor in the pathogenesis of bone fragility (Seeman, 2002). The delay of menarche and infrequent menstrual cycles decrease estrogen levels during adolescence and decrease peak bone mass (Warren, 1991). Furthermore, Warren et al. (2002) found the age of menarche to be more correlated to stress fracture occurrence than bone mineral density (BMD). Therefore, conditions that delay puberty by delaying the maturation of the Hypothalamic-Pituitary-Gonadal (HPG) axis are associated with delayed skeletal development and may affect long-term bone strength. The purpose of this study was to test the hypothesis that suppression of the HPG axis during pubertal development results in a deficit in bone strength accrual.

Gonadotropin releasing hormone antagonists (GnRH-a) have successfully delayed the onset of puberty in female rats as determined by delayed vaginal opening, lower ovarian weights and lower serum estradiol levels (Roth, 2000). This model offers an opportunity to reproduce an environment of delayed menarche and to investigate the effect on bone strength at a critical time point in bone development. The investigative hypothesis predicts that administration of a GnRH-a initiated prior to the onset of the first estrus cycle would impede the development of cortical bone strength in female rats.

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 GnRH-a (Zentaris GmbH) intraperitoneally (0.2ml) for a 29 day period. The experimental groups were separated into a group that injected 125 µg/dose daily (D1) (n-15), 250 µg/dose daily (D2) (n-15) and 500 µg/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. Animals were sacrificed after the final injection, approximately 50 days of age. Uterine weights were measured. Femoral lengths were measured and then the bones were mechanically tested under 3-point bending at a loading rate of 0.1 mm/s. 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 & DISCUSSION

A delay in the timing of puberty through GnRH antagonist injections was confirmed by the delay in the day of vaginal opening of the experimental groups compared to control (Figure 1). 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 (Table 1).

Figure 1: The cumulative percent of animals with VO is displayed per age (days).

Table 1: Summary of group differences in uterine weights, femur length and body weights at sacrifice. Mean (SD). * p<0.05 versus C

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Uterine Weight (g)</td>
<td>.347 ± .079 ± .084 ± .085 ±</td>
</tr>
<tr>
<td>Femur Length (mm)</td>
<td>28.12 ± 25.28 ± 26.34 ± 24.62 ±</td>
</tr>
<tr>
<td>Body Weight at Sacrifice (g)</td>
<td>± 15.85 ± 20.98 ± 20.49 ± 15.33 ±</td>
</tr>
</tbody>
</table>

A significant decrease in femoral length was found (Table 1). This deficit in femoral length was accompanied by a deficit in bending strength of the femoral cortical diaphysis in the GnRH-a groups (Figure 2). Specifically, peak moment and stiffness were lower in the GnRH-a groups. However, the body weights of the experimental animals were significantly larger than control (17.9%). Body weight is often considered a correlate to bone strength. The bone strength deficit could have resulted from a decrease in the quantity of bone, the structural arrangement of the bone from the diaphyseal center or the composition of the bone.

SUMMARY/CONCLUSIONS

GnRH antagonist administered to animals prior to the first estrus cycle at doses of 125 ug/dose and 500 ug/dose significantly decreased the bone strength in the femur at 50 days of age. The mechanisms and duration of this deficit need further investigation.

REFERENCES

Warren et al. (2002) J Clin Endocrinol Metab 87:3162-3168

ACKNOWLEDGEMENTS

NIH Grant AG19654
PSC-CUNY Grant 64293-00-33
Zentaris GmbH