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Multigenerational exposure to genistein does not increase bone mineral density in rats

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Abstract

Genistein has been shown to prevent bone loss in ovariectomized adult rats. However, the effects of genistein on bone in developing and reproductively-intact rats have not been examined. A large multigenerational experiment involved feeding 0, 5, 100, or 500 ppm genistein in the diet to intact male and female rats from conception until either weaning, postnatal day 140, or continuously for 2 years. Vertebrae (lumbar and caudal) were collected from these animals at necropsy at 2 years of age and subjected to dual-energy x-ray absorptiometry (DXA) scanning to measure bone mineral density (BMD), bone mineral content (BMC), and bone area. Femurs were collected, and length, cross-sectional area, and cortical bone area were measured directly. Serum was collected for measurement of pyridinoline (PYD) and alkaline phosphatase (ALP). BMD was not affected by genistein in any phase of the experiment. In female rats treated continuously with genistein, BMC and bone area were reduced in the 500 ppm group compared to the 5 ppm group in the lumbar vertebrae, and in all treatment groups compared to control in the caudal vertebrae. In both males and females treated continuously, the cross-sectional area of the femur was reduced in rats treated with 500 ppm compared to those treated with 5 ppm. In female rats treated continuously, PYD was higher in the 100 and 500 ppm groups than in the 0 and 5 ppm groups. In conclusion, the effects of genistein on reproductively-intact rats were not dramatic. High dose of genistein throughout the lifespan resulted in decreased bone size, which may reduce the force required to break the bone.
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Keywords: Genistein; Bone development; Bone mineral density; Endocrine disruptors; Rat

Introduction

Osteoporosis is a disease of increasing public health significance, affecting one third of women over 65 and an increasing number of men [1]. The loss of bone that occurs with aging results in fractures, especially of the hip and spine [2]. Up to 20% of hip fractures result in death, while spine fractures are a significant cause of morbidity and deformity [1].

Dietary phytoestrogens are increasingly being used as dietary supplements to reduce adverse effects of menopause [3,4], and under some protocols, soy isoflavones prevent loss of bone mineral in postmenopausal women [5–20]. Similarly, results from estrogen-deficient rodent models suggest that soy and isoflavones have bone sparing effects [4,8,21–25]. Several mechanisms have been implicated in the action of isoflavones, and of genistein in particular. Genistein can have estrogenic effects through interaction with estrogen receptors, particularly ER-β, but also has ligand-independent actions involving growth factors, cell-surface signaling regulation of enzymes such as protein tyrosine kinase, and can modulate the action of endogenous estrogens [26].

Resistance of bone to fracture is determined not only by bone mineral density, but also size and geometry, which are determined during development [27]. Furthermore, most studies involving bone endpoints focus on remodeling changes that occur in bone following the attainment of peak bone mass, although low peak bone mass is an established risk factor for osteoporosis [2]. In both humans and animals, conditions or treatments that

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delay puberty, reduce estrogen synthesis, or reduce estrogen sensitivity have been associated with a reduction in peak bone mass (reviewed in [28]). Endogenous estrogen is responsible for growth plate closure and the cessation of longitudinal bone growth in both females and males [29–31]. Migliaccio et al. [32–34] have determined that perinatal treatment of mice with the synthetic estrogen diethylstilbestrol (DES) induced changes in bone development with effects that last into adulthood, including a sustained increase in mineral apposition rate and decrease in osteoclastic activity. As adults, the DES-treated mice had shorter bones with a higher bone mineral density. Fukazawa et al. [35] also reported that neonatal exposure to DES resulted in shorter bones, but reported decreased osteoblast numbers, implying decreased bone formation. Genistein, which has been referred to as a "selective estrogen receptor modulator of plant origin" [26], could alter bone development.

The rate of osteoporotic hip fracture is lower in Japan than in the United States, although bone mineral density (BMD) is actually lower in Japanese women than in white American women [36]. It has been suggested that Japanese women have fewer fractures because of differences in bone geometry; whether this difference is due solely to genetics or is also influenced by diet during growth and maturation is not known. Studies on the effects of soy in premenopausal women have been inconclusive [37–40], and information on the effect of soy during development is minimal [41]. Given the possible effects of soy isoflavones to affect bone growth and development, and given the significant human exposure to soy isoflavones via soy infant formula and soy-based dietary supplements, this study was undertaken to examine the effects of developmental and life-time exposure to genistein in rats.

Materials and methods

Animals and test article

All procedures were approved by the NCTR Animal Care and Use Committee and performed in an AAALAC-accredited animal facility. Sprague–Dawley rats for the parental (F_0) generation were obtained from the NCTR breeding colony at weaning and housed two per cage until allocated to dose groups, after which time they were singly housed in polycarbonate cages with hardwood chip bedding. Genistein (>99%, Toronto Research Chemicals, Inc., North York, Ontario, Canada) was administered at doses of 0, 5, 100, and 500 ppm in Purina 5K96 chow (PMI Nutrition International, Richmond, IN), which is a modified NIH-31 diet that meets the nutritional specifications of NIH-31 but has had soy and alfalfa, major sources of phytoestrogens, removed and replaced by casein. Water was provided ad libitum.

Experimental design

This project was part of a larger multigenerational experiment, outlined in Fig. 1. For the multigeneration reproductive study, males and females of the original parental generation (F_0) were placed on 5K96 diet at weaning and dosed feed was administered starting on PND 42, approximately 1 month before breeding, and were maintained on dosed feed until termination at PND 140. For breeding, one male was cohabited with one female for 14 days or until a vaginal plug was detected. Subsequent generations (F_1–F_4) were similarly bred. The F_1 and F_2 generations were exposed to the test compound administered in the diet continuously from conception through termination

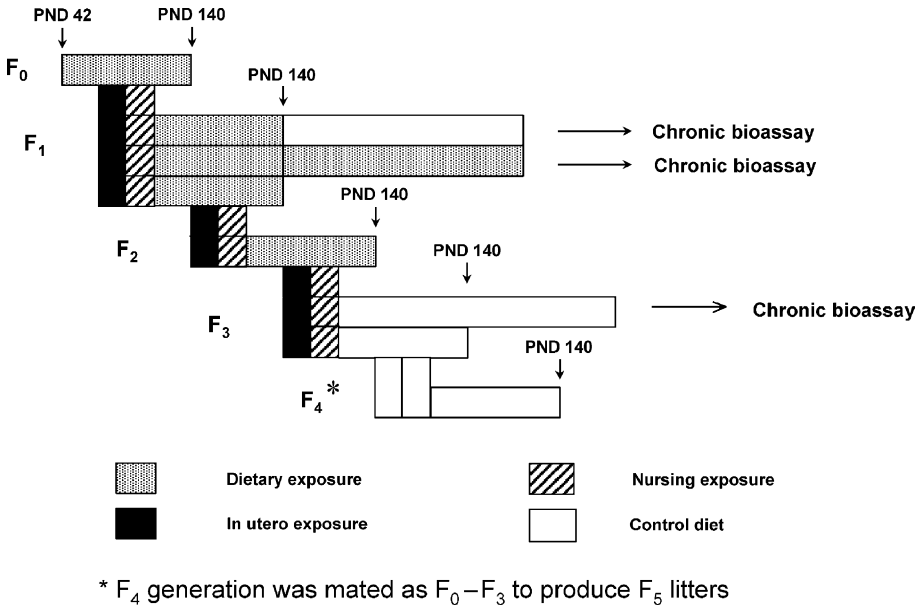

Fig. 1. Multigenerational dosing schedule for parent study. Rats from the chronic bioassays were used for bone assessments.