

Alterations in Estrogen Levels during Development Affects the Skeleton: Use of an Animal Model

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Exposure to estrogens during various stages of development has been shown to irreversibly influence responsive target organs. The recent finding of the presence of estrogen receptor in both osteoblasts and osteoclasts has suggested a direct role of steroid hormones on bone tissue. Furthermore, estrogens have important effects on bone turnover in both humans and experimental animal models. Thus, this tissue is now regarded as a specific estrogen target tissue. To investigate whether a short-term developmental exposure to estrogens can influence bone tissue, we have injected female mice with diethylstilbestrol (DES) from day 1 through day 5 of life. Additionally, a group of pregnant female mice were injected with different doses of DES from day 9 through 16 of pregnancy. Mice were then weaned at 21 days of age, and effects on bone tissue of the female mice were evaluated in adulthood (7–12 months of age). These short-term treatments did not affect body weight of exposed mice. However, a dose-dependent increase in bone mass, both in the trabecular and compact compartments, was observed in the DES-exposed female offspring. Furthermore, femurs from DES-exposed females were shorter than femurs from controls. A normal skeletal mineralization accompanied these changes in the bone tissue. In fact, a parallel increase in total calcium content of the skeleton was found in concomitance with the increase in bone mass. Estrogen treatment induced an increase in the amount of mineralized skeleton when compared to untreated controls. In summary, this report shows that alterations of estrogen levels during development can influence the early phases of bone tissue development inducing permanent changes in the skeleton. These changes appear to be related to bone cell programming in early phases of life. — Environ Health Perspect 103(Suppl 7):95–97 (1995)

Key words: bone tissue, estrogens, development, mice

Introduction

In 1948, Albright et al. (1) were the first to suggest a strong link between the decrease of estrogen levels and the onset of postmenopausal osteoporosis. He had already demonstrated how women develop a severe osteopenia after the reduction of circulating levels of estrogens during the years following menopause (1). Since this first report, many studies have focused on assessing the role estrogens play in bone-cell homeostasis and have demonstrated the importance of these steroids in the onset of postmenopausal osteoporosis (2–6). Additionally, estrogen receptor (ER) has been detected in bone cells, both

osteoblasts and osteoclasts (7–9), and several studies have shown that estrogens can modulate bone cell physiology *in vitro* by a direct ER-mediated mechanism (10–12). This evidence implicates a direct effect of estrogen on the skeleton and ultimately on bone tissue turnover. Natural estrogens such as estradiol were shown to induce permanent changes in the skeleton of adult animals when given as long-term treatment (2–5). Additionally, it has also been shown that synthetic estrogenic compounds such as diethylstilbestrol (DES) can also produce effects on the skeleton of both mice and rats at adulthood (13–15). Furthermore, additional evidence has shown that phytoestrogens, which are widely present in our environment (16–18), seem to have some modulatory effects on bone cells *in vitro* (19) and, moreover, influence skeletal tissue *in vivo* (JB Anderson et al., unpublished data). Finally, exposure to the exogenous estrogenic compound DES during development can influence the growth of estrogen target tissues and produce permanent alterations still detectable in the adult animals (20,21). To evaluate whether changes in the estrogen levels during

development could affect skeletal tissue, a poorly characterized estrogen target organ, a developmental animal model, was used to demonstrate any potential effects.

Materials and Methods

To address the question of whether changes in estrogen levels during development could affect skeletal characteristics, we used an animal model involving neonatal exposure (20) that has been used by several investigators to evaluate the potential effects that alterations of environmental estrogens could have on specific target tissues (22). Outbred CD-1 female mice were injected subcutaneously with DES (2 µg/pup/day) dissolved in corn oil or vehicle only (CTL) from days 1 through 5 after birth as previously described (22). Additionally, pregnant female mice were injected from day 9 through day 16 of pregnancy with different doses of DES in corn oil (0.1–100 µg/kg maternal body weight) or vehicle only (S Migliaccio and KS Korach, unpublished observations). Offspring of the treated pregnant mice or neonatally treated mice were then evaluated at adulthood between 7 and 14 months of age (23).

This paper was presented at the Symposium on Estrogens in the Environment, III: Global Health Implications held 9–11 January 1994 in Washington, DC. Manuscript received: March 15, 1995; manuscript accepted: April 4, 1995.

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Abbreviations used: ER, estrogen receptor; DES, diethylstilbestrol; TRAP, tartrate-resistant acid phosphatase.

Results and Discussion

When animals were evaluated in adulthood, no changes in body weight were found. However, when physical, biochemical, and histological characteristics of bone tissue were estimated, significant differences were found between control and DES-treated animals [S Migliaccio and KS Korach, unpublished observations (22)]. In particular, transient treatment of animals with estrogens during early phases of development, either before birth or soon after birth, enhanced bone mass. This increase was observed both in the cortical and trabecular bone compartments (Figure 1), with similar characteristics observed in animals transiently exposed to the synthetic estrogenic compound either prenatally or neonatally. In particular, the increase in the bone density observed in the prenatally treated mice was dose dependent, suggesting similarities to a receptor-mediated effect. The similar effects observed with both types of treatment suggest that a period of sensitivity exists during which estrogens can influence bone tissue development.

To further investigate the mechanism(s) involved in this event and whether these changes could be the result of different bone cell homeostasis, analysis of osteoclastic cells was performed. Evaluation was performed using the tartrate-resistant acid phosphatase (TRAP) activity as a marker for osteoclast activity. Our results suggest a decrease in osteoclast number and TRAP activity in the animal developmentally treated with DES (S Migliaccio and KS Korach, unpublished observations). The presence of a change in the osteoclast number and TRAP activity in the bone tissue of these adult female mice strongly suggests that the transient treatment with estrogen during development influenced bone cell programming with a long-term effect detectable in later phases of life. The changes in the bone cell activity of these animals seem to suggest a decrease in the

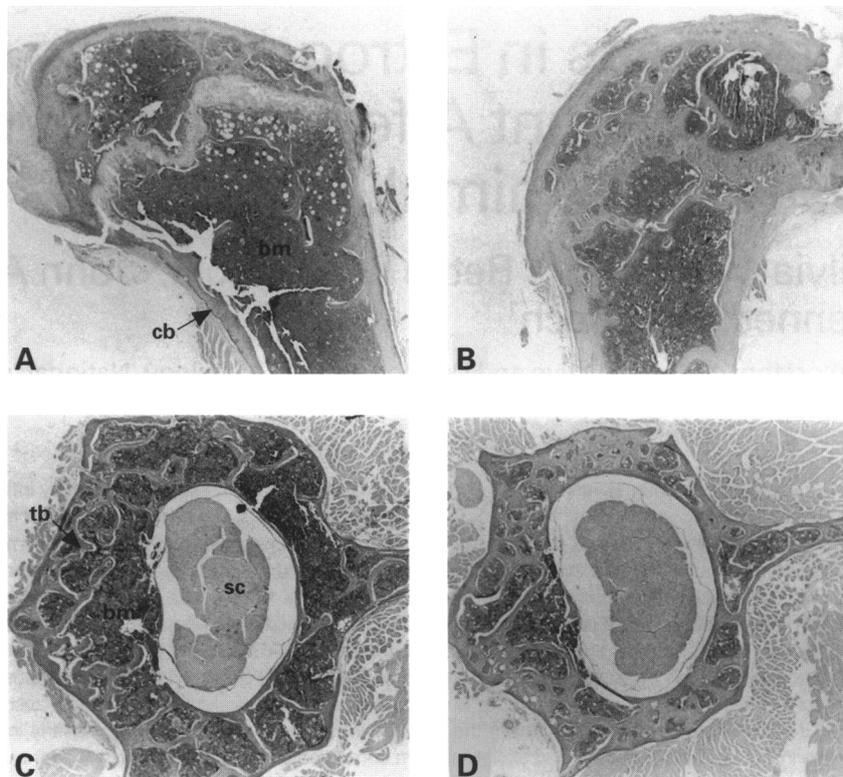


Figure 1. Bone specimens from control and DES-treated animals. Longitudinal section of distal femur from (A) control and (B) DES-treated mice. Cross-section of a lumbar vertebra from (C) control and (D) DES-treated animals. Bone sections were stained with hematoxylin and eosin. Abbreviations: tb, trabecular bone; bm, bone marrow; cb, compact bone; sc, spinal cord.

amount of the bone resorption, with a final result of an increase in the bone mass.

At the present time our result cannot either indicate or rule out any potential teratogenic or carcinogenic effect of the early exposure to DES in the skeleton of these female mice. It is interesting to know, however, that there have been a few self reports of spondylolisthesis in women exposed prenatally to DES (23). Thus, it may be clinically important to further investigate the possibility that exposure of the human population to exogenous estrogens could permanently affect skeletal

tissue. In particular, it will be clinically relevant to evaluate whether low or high doses of these steroids could affect the skeleton in different manner, for instance increasing peak bone density or inducing skeletal malformations at higher doses. In conclusion, our results show for the first time that developmental exposure to estrogens during certain stages can permanently influence bone tissue in an animal model. The observed changes appear to be due to an effect on bone cell programming since differences in bone cell number and activity are retained through adulthood.

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