J. Endocrinol. Invest. 34: 106-110, 2011
DOI: 10.3275/7108

The effects of native and synthetic estrogenic compounds as well as vitamin D less-calcemic analogs on adipocytes content in rat bone marrow

D. Somjen[1], S. Katzburg[1], F. Kohen[2], B. Gayer[2], G.H. Posner[3], I. Yoles[4], and E. Livne[5]

[1]Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Centre, and The Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv; [2]Department of Biological Regulation, The Weizmann Institute of Science Rehovot, Israel; [3]Department of Chemistry, The Johns Hopkins University, Baltimore, MD, USA; [4]Department of Gynecology, Tel-Hashomer Medical Center and The Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv; [5]Department of Anatomy and Cell Biology, Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

ABSTRACT. ***Background:*** **We demonstrated previously that phytoestrogens and vitamin D analogs like estradiol-17β (E_2) modulate bone morphology in rat female model.** ***Aim:*** **We now analyze the effects of phytoestrogens, E_2, selective E_2 receptor modulators, and the less-calcemic analogs of vitamin D: JKF1624F_2-2 (JKF) or QW1624F_2-2 (QW) on fat content in bone marrow (BM) from long bones in ovariectomized female rats (OVX).** ***Materials and methods:*** **OVX rats were injected with treatments known to affect bone formation, 5 days per week for 2.5 month for analysis of fat content in BM.** ***Results:*** **In OVX young adults there is a decreased bone formation and a 10-fold increase in fat cells content in BM. Treatment with E_2, raloxifene (Ral) or DT56a resulted in almost completely abolishment of fat cells content. Daidzein (D) decreased fat cells content by 80%, genistein (G) or biochainin A (BA) did not change fat cells content and carboxy BA (cBA) had a small but significant effect. JKF or QW did not affect fat cells content, whereas combined treatment of JKF or QW with E_2 resulted in complete abolishment of fat cells content. These changes in fat cells content are inversely correlated with changes in bone formation.** ***Conclusions:*** **Our results demonstrate that adipogenesis induced by OVX is a reversible process which can be corrected by hormonal treatments. The awareness of a relationship between fat and bone at the marrow level might provide a better understanding of the pathophysiology of bone loss as well as a novel approach to diagnosis and treatment of postmenopausal osteoporosis.**

(J. Endocrinol. Invest. 34: 106-110, 2011)

INTRODUCTION

Osteoporosis is associated with atrophy of the spongy bone due to reduction in bone formation, thus affecting bone strength to the extent that fractures occur after minimal trauma. At menopause, an accelerated loss of bone mass (3%/yr) takes place during the first 5 yr, along with an increased volume of bone marrow (BM) adipocytes.

Previous histomorphometric studies showed an association between osteopenia and increased adipose tissue in BM with aging (1, 2). Such a phenomenon was observed also in ovariectomized (OVX) rats (3). In humans, osteoporosis and age-related osteopenia were shown to be associated with an increase in marrow fat tissue. It was shown that the number of osteoblasts were negatively correlated with the numbers of adipocytes (4), suggesting that adipocytes were generated at the expense of osteoblasts. The increased numbers of adipocytes observed in BM in osteoporosis and age-related bone loss may be directly linked to osteogenic potential, reflecting a switch in the balance of commitment of stem cells from osteoblasts to adipocytes (5, 6). Following ovariectomy, bone volume in rat metaphysis decreased and the space was filled with hemopoietic and adipose tissue. Marrow fat content increased with time after ovariectomy, with a reciprocal relationship between marrow fat content and bone formation rate (7, 8).

Factors expected to enhance osteogenesis enhance also adipogenesis and *vice versa*, *via* transcription factors and cytokines that serve as mediators for terminal differentiation of both (9).

Estrogen deficiency is known to be involved in osteoporosis (10), and it has been reported that estrogens as well as androgens, are necessary for the maintenance of the male as well as the female skeleton (11, 12). Recently, we have demonstrated that some phytoestrogenic derivatives such as DT56a have estrogen-like activities on bone formation (13, 14) and can replace current hormone replacement therapy (HRT) treatments with no deleterious effects.

We also tested the less-calcemic vitamin D_3 analogs QW1624F_2-2 (QW) and JKF1624F_2-2 (JKF), and found repair of the loss of trabecular bone and the gain of adipcytes in rats long bones occurring immediately after ovariectomy (15, 16).

Ovariectomy was shown to increase body weight by stimulating an increase in carcass adiposity due primarily to increased fat mass, while estrogen treatment prevented this increase in adult OVX rats (17, 18). Whether or not hormonal treatments affecting osteoblasts and bone formation will affect adipocytes number in BM leading to the reversal of this process is not clear.

Key-words: Bone marrow, estradiol-17β, fat cells, phytoestrogens, vitamin D analogs.

Correspondence: D. Somjen, PhD, Institute of Endocrinology, Metabolism and Hypertension Tel-Aviv Sourasky Medical Centre, 6 Weizmann street, Tel-Aviv 64239, Israel.

E-mail: dalias@tasmc.health.gov.il

Accepted February 11, 2010.

First published online June 11, 2010.

Using rat models, it was shown that there is an increased numbers of adipocytes in BM of aged and OVX animals, associated with thinning of structure of trabecular bone due to a reduction in osteoblastic activity (19).
The aim of the present study was to evaluate the effects of different estrogenic compounds as well as vitamin D less-calcemic analogs on BM adipocytes volume (%MAV) in BM of long bones from OVX female rats treated for 2.5 months.

MATERIAL AND METHODS

Animals

In several experiments carried out in our laboratory, Wistar-derived, locally bred female rats, aged 25 days and weighing 60 g at the start of the experiment, were maintained, on a 14 h light/10 h dark schedule at 23 C, and provided with food pellets and water *ad libitum*. Experiments were carried out according to the regulations of the Committee on Experimental Animals of the Tel-Aviv Sourasky Medical Center and the NIH guidelines. Ovariectomy was carried out at 25 days of age and used starting 2 weeks post-surgery.

Hormonal treatment

Animals were divided into groups (no.=5 for each treatment group) and different experiments were used: a) intact, OVX, and OVX animals treated with estradiol-17β (E_2) and DT56a; b) intact, OVX, and OVX animals treated with E_2, raloxifene (Ral), genistein (G), daidzein (D), biochainin A (BA), or carboxy BA (cBA); c) intact, OVX, and OVX animals treated with QW, JKF, E_2 or E_2 + JKF and E_2 + QW (14-16).
In all studies, OVX rats were injected 5 days per week with: a) 166 µg/kg E_2, with 650 µg DT56a, with 1660 µg/kg D or with vehicle 0.1% ethanol in saline (C); b) 166 µg/kg E_2, 1660 µg/kg Ral, 1660 µg/kg G, D, cBA or BA, or with vehicle 0.1% ethanol in saline (C); c) with E_2 (1 µg/rat), with JKF or QW at 0.2 ng/g BW or JKF or QW at 0.2 ng/g BW followed by injections of E_2 (1 µg/rat) on day 5 of each week, or with 0.1% ethanol in saline (C) (15, 20).

Histology and histomorphometry

After 2.5 months of treatment, 24 h after the last injection, rats were sacrificed and organs were removed for histomorphometry. Tibiae were dissected and fixed in 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2, and decalcified in 10% EDTA in 0.05M Tris-HCl buffer, pH 7.2. Specimens were embedded in paraffin and 6-mm thick sections, parallel to the long axis of the bone, were cut serially and stained with hematoxylin and eosin. The %MAV was determined in a defined area, directly under the tibial growth plate. Image capture, processing and analysis were done using Image Pro Plus software, a computerized histomorphometric system (Media Cybernetics Inc. USA).

Statistical analysis

Statistical analysis was carried out using the Stat-2 software. The data were subjected to one way analysis of variance (Kruskal-wallis test) and TUKEY non-parametric test for differences between groups.

RESULTS

In all experiments, specimens were studied at the metaphysis including the growth plate and the primary spongiosa adjacent to the growth plate.
Examination of the control intact animal group revealed that the growth plate contained the typical arrangement of cartilage cells, including proliferative, chondroblastic, and hypertrophic chondrocytes. Numerous thin trabecular spicules were observed underneath and adjacent to the lower aspect of the growth plate and adipocytes were scarcely observed (Fig. 1 and Table 1).
After 2.5 months from ovariectomy, control animals demonstrated changes in the growth plate structure. The overall growth plate architecture was disrupted with fewer proliferative and chondroblastic cells. The metaphysis underneath the growth plate contained only few trabeculas as compared to the intact control tibiae. Unlike the BM appearance of bone from intact rats, the metaphysis from OVX rats contained plenteous adipocytes, and %MAV was 27.10±1.80% as compared to 0.28±0.17% in the intact control (Fig. 1 and Table 1).

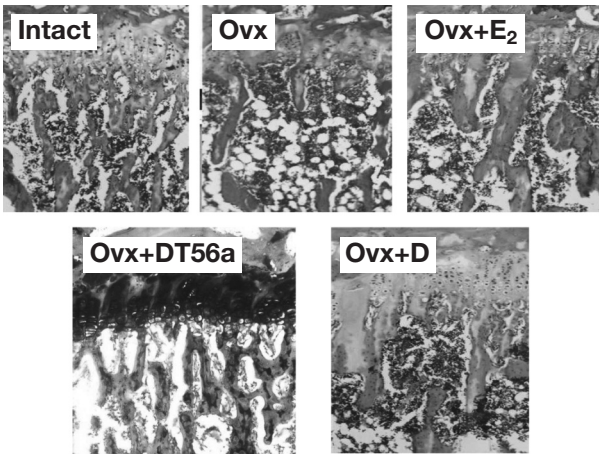

Fig. 1 - *The effects of estradiol 17β (E_2), DT56a, and daidzein (D) on fat volume in bone marrow from ovariectomized (OVX) female rats. Details are as described in Materials and Methods.*

Effects of E_2, DT56a, and D on bone marrow fat volume

After 2.5 month of treatment with E_2, the tibial morphological appearance was similar to that observed in the intact bone. Treatment with E_2 restored bone loss in the OVX rats by 32% compared with the control OVX rats ($p<0.01$) (14-16). The growth plates contained the typical arrangement of proliferative, chondroblastic, and hypertrophic cellular populations. The bony trabeculae observed in the primary spongiosa appeared to be thicker

Table 1 - *The effects of estradiol 17β (E_2), DT56a, and daidzein (D) on fat volume (FV) in bone marrow.*

	%FV in bone marrow (%FV±SEM)
Control intact	0.28±0.17
OVX + Control	27.10±1.80[a]
OVX + E_2	0.03±0.002[a]
OVX + DT56a	0.00±0.00[a]
OVX + D	5.37±0.30[b]

[a]$p<0.01$, [b]$p<0.05$, from the corresponding mean values of ovariectomized control rats.

as compared to the spicules observed in the intact control animals whereas the %MAV was reduced by almost 100% from 27.10±1.80% to 0.03±0.002% ($p<0.01$) (Fig. 1 and Table 1). The growth plate architecture has been partially restored after treatment with D and the primary spongiosa contained thick bone trabecules with a reduction of 80% in %MAV from 27.10±1.80% to 5.37±0.30% ($p<0.05$) (Fig. 1 and Table 1).
DT56a treatment completely prevented the OVX-induced reduction of growth plate width, restoring the growth plate to the same width as that in non-OVX controls. Moreover, the growth plates of DT56a-treated rats were more mature in appearance than those of E2-treated rats, containing relatively more chondroblastic and hypertrophic cells and fewer proliferative cells. Treatment with DT56a decreased completely and significantly the %MAV from 27.10±1.80% to 0.00±0.00% ($p<0.01$) (Fig. 1 and Table 1) (14, 16).

Effects of several estrogenic compounds on bone marrow fat volume

Treatment of OVX rats for 2.5 months with Ral revealed an almost complete recovery of the bone architecture. The growth plate arrangement was typical and normal and there was an increased % trabecular bone volume (%TBV), restoring bone loss caused by ovariectomy (16). A complete reduction in %MAV was observed in this region from 27.10±1.80% to 0.20±0.02% (Fig. 2 and Table 2). A disrupted organization of the growth plate and trabecules was observed after treatment for the same time with G. No proliferative chondrocytes were observed. Most of the growth plate contained chondroblastic and hypertrophic chondrocytes. G only slightly decreased %MAV from 27.10±1.80% to 23.00±2.10 % (Fig. 2 and Table 2). Compared to the OVX control rats, no change was observed after treatment with BA with BM that contained large %MAV, similar to that observed in the OVX control rats from 27.10±1.80% to 25.07±1.90% (Fig. 2 and Table 2). Similar results were observed after treatment with cBA with 30% decrease in %MAV from 27.10±1.80% to 19.10±1.60% (ns) (Fig. 2 and Table 2) (16).

Table 2 - *The effects of different estrogenic compounds on fat volume (FV) in bone marrow.*

	%FV in bone marrow (%FV±SEM)
Control intact	0.28±0.17
OVX Control	27.10±1.80[a]
OVX + E_2	0.03±0.002[a]
OVX + DT56a	0.00±0.00[a]
OVX + Ral	0.20±0.02[a]
OVX + D	5.37±0.30[b]
OVX + G	23.00±2.10
OVX + BA	25.07±1.90
OVX + cBA	19.10±1.60

[a]$p<0.01$, [b]$p<0.05$, from the corresponding mean values of ovariectomized (OVX) control rats. E_2: estradiol 17β; Ral: raloxifene; D: daidzein; G: genistein; BA: biochainin A; cBA: carboxy BA.

Effects of less-calcemic vitamin D analogs: JKF and QW on bone marrow fat volume

Treatment of 2.5 month with JKF resulted in some recovery of the bone architecture of OVX rats, but not similar to bone from intact rats, along with a thin epiphiseal cartilage with not many proliferative cells, vast hypertrophic cells. A significant difference was found in cortical bone thickness and %TBV, and numerous adipocytes in the bone marrow (15, 20). In fact, JKF increased the MAV by 120% from 32% to 70% (Fig. 3 and Table 3).
Addition of E_2 to the treatment with JKF restored some aspects of bone architecture. Hardly any bone marrow adipocytes were observed with 100% reduction of %MAV from 32 to 0% (Fig. 3 and Table 3) (15, 20).
Treatment of OVX rats with QW restored the growth

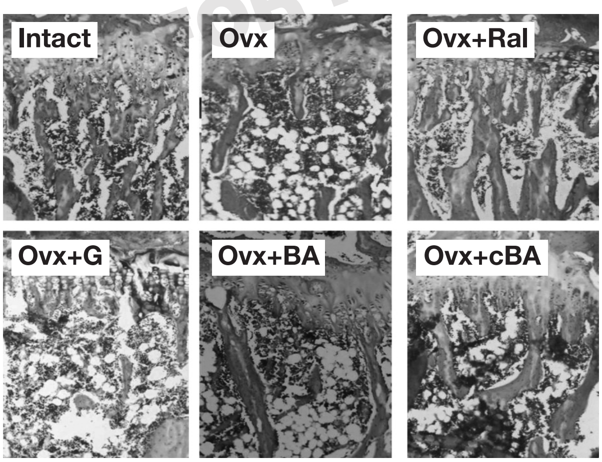

Fig. 2 - *The effects of raloxifene (Ral), genistein (G), biochainin A (BA) and carboxy BA (cBA) on fat volume in bone marrow from ovariectomized (OVX) female rats. Details are as described in Materials and Methods.*

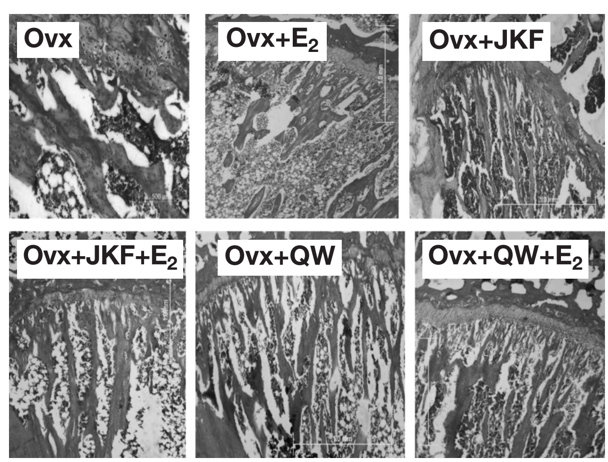

Fig. 3 - *The effects of less-calcemic vitamin D analogs JKF1624F$_2$-2 (JKF) and QW1624F$_2$-2 (QW) on fat volume in bone marrow from ovariectomized (OVX) female rats. Details are as described in Materials and Methods. E_2: estradiol 17β.*

Table 3 - *The effects of less-calcemic vitamin D analogs JKF1624F$_2$-2 (JKF) and QW1624F$_2$-2 (QW) on fat volume (FV) in bone marrow.*

	%FV in bone marrow (%FV)
Control intact	0.28
OVX Control	32.00
OVX + E_2	0.48
OVX + JKF	70.00
OVX + JKF + E_2	0.00
OVX + QW	30.00
OVX + QW + E_2	0.00

E_2: estradiol 17β.

plate structure, however with slight disruption of its organization. Proliferative cells were observed and although the mineralized zone appeared to be resorbed, it was not accompanied with trabecular bone formation or with less adipocytes in bone marrow. %MAV was reduced slightly from 32 to 30% (Fig. 3 and Table 3). Addition of E_2 to the treatment of QW resulted in a complete restoration of the growth plate architecture displaying normal appearance with the typical division of the growth plate zone and thin spicules. Moreover, the primary spongiosa contained thin bone trabeculae, %TBV was restored to that observed in the intact control rats, and almost no adipocytes in bone marrow were observed, with a reduction of 100% in MAV from 32 to 0% (Fig. 3 and Table 3) (15, 20).

DISCUSSION

In the present studies, the effect of various phytoestrogens, both native and synthetic, E_2 and Ral on rat bone, as well as the less-calcemic 1,25(OH)$_2$D$_3$ analogs JKF and QW were compared and analyzed regarding their ability to repair the damage caused by ovariectomy. Ovariectomy caused an almost complete cessation of bone growth, as was demonstrated by the disruption of the growth plate cell organization, and the complete disappearance of thin bone spicules, with an increased amount of adipocytes replacing osteoblasts in the bone marrow of long bones adjacent to the growth plate (14-16, 20).

We demonstrated that the parameter of bone marrow adipocytes, i.e. %MAV increased due to ovariectomy, and that E_2, DT56a, D, Ral, and combined treatment of QW+E_2 or JKF+E_2 were able to reverse this increase. Thus, indicating that these hormonal treatments promote osteogenic formation, replacing the vast medullary adipocytes due to the removing of the source of estrogens by ovariectomy. These results may indicate that the hormonal treatments affected overall BM metabolism.

The changes caused by OVX reflects a more mature stage of bone as observed in aged animals such as mice with accelerated aging (SAMP6 strain), osteoblastogenesis was decreased with correlation to increased number of adipocytes (21). Age-related changes in the differentiation potential of mesenchymal marrow stem cells (mMSC) are accompanied by alterations in intracellular mechanisms and extracellular signaling controlling their fate (22). With aging, the levels of stimulators of bone formation such as transforming growth factor (TGF)-β and interleukin (IL)-11 are decreased, and inhibitors of bone formation such as tumor necrosis factor (TNF)-α and IL-6 are increased (23, 24). In addition, the expression of adipocyte-restricted peroxisome proliferator-activated receptor-γ2 transcription factor, which is a key regulator of osteoblasts and adipocytes differentiation, is increased in marrow from old mammals (25).

Clinical studies have shown that decreased bone mass is inversely correlated with increased BM fat content, and increased marrow fat contents secretes large amounts of TNFα and IL-6, which in turn inhibit osteoblastogenesis (26). Other clinical observations documented an inverse relationship between adipocytes and osteoblasts in osteoporotic patients (27).

In our study, we found that treatment with E_2 as well as with some of the estrogenic compounds reversed the aging process of BM by reducing its lipid content and repaired the morphology of the growth plate. These results are in accordance with studies examining the effect of estrogens on BM from OVX mice on adipogenesis, showing that old animals responded better to E_2 administration by reducing the high content of marrow fat in their bones (28).

Magnetic resonance imaging revealed that the effect of ovariectomy on rat fat was not limited to subcutaneous and abdominal fat, but included fat composition of marrow ovariectomy and induced an increase in the fat content of the rat tibial metaphyses. Furthermore, treatment with E_2 was correlated with a decrease in tibial marrow fat *in vivo* after 1 week of treatment (18).

Using a murine pre-osteoblastic clonal cell line KS483 it was shown that E_2 stimulates the differentiation of progenitor cells into osteoblasts and concurrently inhibits adipocyte formation in an estrogen receptor (ER)-dependent way (29). Estrogen may recruit progenitor cells in the osteoblastic lineage at the expense of adipocytes and it may stimulate maturation of early osteoblasts as was demonstrated in both KS483 and ST-2 stromal cell lines (29).

Studying the effect of several phytoestrogens on bone, we found that G, BA or cBA had almost no effect on the OVX rats bone structure (16) and increased amounts of adipocytes were observed in BM similar to BM from untreated OVX rats. Only DT56a and D promoted osteoblastic formation, replacing the extensive numbers of adipocytes caused by ovariectomy (14, 16).

In vitro studies of the soy pyhtoestrogens D (30) and G (31) showed that at low concentrations, both D and G have estrogenic effects, stimulating osteoblastogenesis and decreasing adipogenesis. It was shown that in human BM stromal cells G enhanced the commitment and differentiation of BM stromal cells to the osteoblastic lineage with no effect on the late osteogenic maturation markers. Conversely, G as well as E_2 reduced adipogenic differentiation and maturation (31). However, while *in vitro* studies on human cultures of bone cells showed that G was as active as D or E_2 (31), in our *in vivo* studies D partially restored bone formation including disappearance of BM adipocytes, indicating that aside from its effect on bone growth and maturation, this substance may have also an effect on BM metabolism, while G had no effect (Table 2). Our findings on the effect of G (16) are in

contrast with other studies *in vivo* showing that administration of G decreased bone loss in OVX rats (32) and mice (33), but in accordance with the *in vitro* studies where G is as active as D or E_2 (34).
When the selective ER modulator Ral was administered, there was almost complete recovery of bone morphology, with no adipocytes observed in BM (Table 2). Treatment with the less-calcemic vitamin D_3 analogs JKF and QW restored a few aspects of the OVX bone architecture (16), however, an increased number of BM adipocytes were observed compared to OVX rats. The addition of E_2 to JKF or QW, improved all bone parameters examined (15, 20) including complete disappearance of the adipocytes in BM of long bones (Table 3).
Our present study showed that adipogenesis caused by ovariectomy is a reversible process, which can be corrected and may rejuvenate BM to its normal accurate chronological age by the addition of some of the hormonal compounds tested.

REFERENCES

1. Burkhardt R, Kettner G, Bohm W, et al. Changes in trabecular bone, hematopoiesis and bone marrow vessels in aplastic anemia, primary osteoporosis, and old age: a comparative histomorphometric study. Bone 1987, 8: 157-64.
2. Meunier P, Aaron J, Edouard C, Vignon G. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. Clin Orthop Relat Res 1971, 80: 147-54.
3. Wronski TJ, Dann LM, Scott KS, Cintrón M. Long-term effects of ovariectomy and aging on the rat skeleton. Calcif Tissue Int 1989, 45: 360-6.
4. David V, Martin A, Lafage-Proust MH, et al. Mechanical loading down-regulates peroxisome proliferator-activated receptor gamma in bone marrow stromal cells and favors osteoblastogenesis at the expense of adipogenesis. Endocrinology 2007, 148: 2553-62.
5. Rozman C, Feliu E, Berga L, Reverter JC, Climent C, Ferrán MJ. Age-related variations of fat tissue fraction in normal human bone marrow depend both on size and number of adipocytes: a stereological study. Exp Hematol 1989, 17: 34-7.
6. Compston JE. Bone marrow and bone: a functional unit. J Endocrinol 2002, 173: 387-94.
7. Martin RB, Zissimos SL. Relationships between marrow fat and bone turnover in ovariectomized and intact rats. Bone 1991, 12: 123-31.
8. Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol 2000, 16: 145-71.
9. Chan GK, Duque G. Age-related bone loss: old bone, new facts. Gerontology 2002, 48: 62-71.
10. Pacifici R. Cytokines, estrogen and postmenopausal osteoporosis--the second decade. Endocrinology 1998, 139: 2659-61.
11. Riggs BL, Khosla S, Melton III LJ. A unitary model for involutional osteoporosis: Estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 1998, 13: 763-73.
12. Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 2002, 23: 279-302.
13. Somjen D, Yoles I. DT56a (Tofupill/Femarelle) selectively stimulates creatine kinase specific activity in skeletal tissues of rats but not in the uterus. J Steroid Biochem Mol Biol 2003, 86: 93-8.
14. Somjen D, Katzburg S, Livne E, Yoles I. DT56a (Femarelle) stimulates bone formation in female rats. BJOG 2005, 112: 981-5.
15. Somjen D, Katzburg S, Posner GH, Livne E, Kaye AM. Systemic treatments with the low-calcemic 1,25(OH)(2)D(3) analogs JKF or QW increaseboth the morphological and biochemical responses to estradiol-17β in rat tibiae. J Cell Biochem 2007, 100: 1406-14.
16. Somjen D, Katzburg S, Kohen F, Gayer B, Livne E. Daidzein unlike other isolated isoflavones, preserves bone architecture in ovariectomized female rats in vivo. J Cell Biochem 2007,103: 1826-32.
17. Sato M, Rippy MK, Bryant HU. Raloxifene, tamoxifen, nafoxidine or estrogen effects on reproductive and nonreproductive tissues in ovariectomized rats. FASEB J 1996, 10: 905-12.
18. Sharp JC, Copps JC, Liu Q, et al. Analysis of ovariectomy and estrogen effects on body composition in rats by X-ray and magnetic resonance imaging techniques. J Bone Miner Res 2000, 15: 138-46.
19. Benayahu D, Shur I, Ben-Eliyahu S. Hormonal changes affect the boneand bone marrow cells in a rat model. J Cell Biochem 79, 2000: 407-15.
20. Somjen D. Vitamin D modulation of the activity of estrogenic compounds in bone cells in vitro and in vivo. Crit Rev Eukaryot Gene Expr 2007, 17: 115-47.
21. Kajkenova O, Lecka-Czernik B, Gubrij I, et al. Increased adipogenesis and myelopoiesis in the bone marrow of SAMP6, a murine model of defective osteoblastogenesis and low turnover osteopenia. J Bone Miner Res 1997,12: 1772-9.
22. Moore SG, Dawson KL. Red and yellow marrow in the femur: age-related changes in appearance at MR imaging. Radiology 1990, 175: 219-23.
23. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. Aging Cell 2004, 3: 379-89.
24. Gazit D, Zilberman Y, Ebner R, Kahn A. Bone loss (osteopenia) in old male mice results from diminished activity and availability of TGF-β. J Cell Biochem 1998, 70: 478-88.
25. Lecka-Czernik B, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. Endocrinology 2002,143: 2376-84.
26. Gimble JM, Robinson CE, Wu X, Kelly KA. The function of adipocytes in the bone marrow stroma: an update. Bone 1996, 19: 421-8.
27. Kirkland JL, Tchkonia T, Pirtskhalava T, Han J, Karagiannides I. Adipogenesis and aging: does aging make fat go MAD? Exp Gerontol 2002, 37: 757-67.
28. Elbaz A, Rivas D, Duque G. Effect of estrogens on bone marrow adipogenesis and Sirt1 in aging C57BL/6J mice. Biogerontology 2009, March 31 [Epub ahead of print]; doi: 10.1007/s10522-009-9221-7.
29. Dang ZC, van Bezooijen RL, Karperien M, Papapoulos SE, Löwik CW. Exposure of KS483 cells to estrogen enhances osteogenesis and inhibits adipogenesis. J Bone Miner Res 2002, 17: 394-405.
30. Dang ZC, Lowik CW. Differential effects of PD98059 and U0126 on osteogenesis and adipogenesis. J Cell Biochem 2004, 92: 525-33.
31. Heim M, Frank O, Kampmann G, et al. The phytoestrogen genistein enhances osteogenesis and represses adipogenic differentiation of human primary bone marrow stromal cells. Endocrinology 2004, 145: 848-59.
32. Somjen D, Katzburg S, Kohen F, et al. Responsiveness to phytoestrogens in primary human osteoblasts is modulated differentially by a "less-calcemic" analog of 1,25 dihydroxyvitamin D(3): JK 1624F(2)-2 (JKF). J Steroid Biochem Mol Biol 2006, 98: 139-46.
33. Fanti P, Monier-Faugere MC, Geng Z, et al. The phytoestrogen genistein reduces bone loss in short-term ovariectomized rats. Osteoporos Int 1998, 8: 274-81.
34. Ishimi Y, Miyaura C, Ohmura M, et al. selective effects of genistein, a soybean isoflavone, on b-lymphopoiesis and bone loss caused by estrogen deficiency. Endocrinology 1999, 140: 1893-900.