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Bone Loss in Ovariectomized Rats: Dominant Role for Estrogen But Apparently Not for FSH

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ABSTRACT

Estrogen deficiency as the sole factor underlying post-menopausal osteoporosis was challenged, in light of reports that both follicular stimulation hormone (FSH) receptor and FSHβ knockout mice were resistant to bone loss, suggesting a detrimental role for FSH. We assessed whether lowering FSH levels by gonadotropin realizing (GnRH) analog decapeptyl in ovariectomized female rats (OVX) affects bone. Wistar-derived 25 days old OVX female rats were injected for 10 weeks with estradiol-17β (E_2), with GnRH analog (decapeptyl) or with both. FSH and luteinizing hormone (LH) serum levels were markedly increased in OVX rats, with smaller growth plates with disrupted architecture; heavy infiltration of bone marrow with numerous adipocytes and reduced thickness of cortical bone. In OVX rats treated with E_2, FSH, and LH levels were intermediate, the tibia was similar to that of intact rats, but there was reduced thickness of cortical bone. In decapeptyl treated OVX rats, FSH and LH levels were suppressed, the organization of growth plate and the trabecular bone were disrupted, and there were fewer proliferative and chondroblastic cells and a large adipocytes population in bone marrow, but an increased trabecular bone volume (TBV). In the E_2 + decapeptyl treatment, FSH and LH levels were suppressed, with partially restored growth plate architecture and improved TBV. In conclusion, E_2 deficiency is the dominant factor impairing bone loss in OVX and concomitant changes in FSH/LH levels achieved by decapeptyl have some modulating, though complex role in this setting. The role of high FSH levels in post-menopausal bone loss requires further investigation using combined sub-optimal doses of the different hormones. J. Cell. Biochem. 112: 128–137, 2011.

KEY WORDS: OVARIECTOMIZED FEMALE RATS; LUTEINIZING HORMONE; FOLLICULAR STIMULATING HORMONE; ESTRADIOL-17β

Estrogen deficiency has been traditionally considered to be the sole culprit in hypogonadal bone loss [Albright et al., 1941] and evidence accumulated in the course of more than two decades overwhelmingly indicates that estrogen replacement therapy prevents this loss [Riggs et al., 2002]. There is evidence that estrogen inhibits osteoclastic bone resorption by reducing the release of the inflammatory cytokines IL-1, IL-6, and TNF-α from osteoblasts. Estrogen alters the secretion of the critical osteoclastogenic cytokine RANK-L and its decoy receptor osteoprotegerin (OPG) from osteoblasts, thereby affecting osteoclast formation [Srivastava et al., 2001]. Estrogen also directly inhibits osteoclast differentiation by acting on bone marrow cells precursors [Srivastava et al., 1998; Shevde et al., 2000].

Interestingly, during the menopausal transition, despite no reduction in circulating estrogen, and in the presence of elevated follicular stimulation hormone (FSH) levels, there is a substantial increase in the rate of bone loss [Sowers et al., 2003, 2006]. The established view of estrogen deficiency causing post-menopausal osteoporosis has been challenged by the recent proposal that FSH is required for hypogonadal bone loss, and that high levels of FSH is the cause of this condition [Sun et al., 2006]. This conclusion emerged from studies of FSH receptor (FSHR) knockout and FSHβ knockout mice, which were reportedly resistant to bone loss despite severe estrogen deficiency. FSHR protein was detected in osteoclasts, and FSH increased osteoclastogenesis and dentine resorption in vitro, but did not influence bone formation or osteoblast activity directly [Gao et al., 2007]. Important criticism and questions have emerged and these findings are still the subject of much debate [Baron, 2006; Martin and Gaddy, 2006; Seibel et al., 2006; Gao et al., 2007; Williams, 2007]. Hence, the regulation of bone metabolism by the hypothalamic–pituitary–gonadal axis and particularly the contribution of high FSH levels to menopausal bone loss need to be defined with greater clarity.

Decapeptyl SR contains the active ingredient Triptorelin acetate, which is a GnRH analog. GnRH acts on its receptors in the pituitary gland, causing the release of LH and FSH and hence the subsequent

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production of testosterone in men and estrogen in women. Triptorelin is a synthetic super active analog of GnRH which acts on the GnRH receptors in the pituitary gland in the same way as the natural neuropeptide. Initially, Triptorelin causes an increase in the amount of FSH and LH released from the pituitary gland, thus resulting in increase in testosterone production in men, and estrogen production in women. However, chronic administration of Triptorelin desensitizes the pituitary gland so that within several days the release of FSH and LH ceases and consequently the production of estrogens in women and testosterone in men decline dramatically.

In the present study we assessed whether or not lowering FSH and LH levels by the use of the GnRH analog in ovariectomized (OVX) female rats, by itself or in combination with estrogen, might improve bone quality.

MATERIALS AND METHODS

ANIMALS

Forty Wistar-derived, female rats, aged 25 days weighing 60 ± 3 g, were maintained on a 14 h light/10 h dark schedule at 23°C. Pelleted food and water were provided ad libitum. The rats were divided into five equal groups: intact, ovariectomized (OVX), and three groups of OVX treated with either estradiol-17β (E_2) or GnRH analog (decapeptyl) or decapeptyl + E_2. All experiments were carried out in compliance with the NIH guidelines and of the regulations of the Committee on Experimental Animals of the Tel-Aviv Sourasky Medical Center, using accepted standards of human animal care.

HORMONAL TREATMENT

Starting 2 weeks post-surgery [Ornoy and Katzburg, 1995; Somjen et al., 2006] OVX female rats were injected 5 days per week, for 10 weeks with 0.5 μg E_2 i.p. or with 3.75 μg decapeptyl SR (Ferring GmbH, Kiel, Germany, was kindly provided by Lapidot Pharmaceuticals Ltd., Caesarea, Israel.) every 10 days (intramuscular) or with decapeptyl + E_2.

HISTOLOGY AND HISTOMORPHOMETRY

After 10 weeks of treatment and 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 0.05 M 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.

Trabecular bone from each treatment group was measured at the proximal tibial metaphysis adjacent to the lower aspect of the growth plate. All measurements were performed in multiple randomized frames for each rat ($n = 5$) of each treatment group. The height of the growth plate, the trabecular (spicules) width underneath the growth plate and the arrangement of cells in the growth plate were also determined. For analysis of cortical bone width, measurements were taken at 50-mm intervals on three sections of the tibial midshaft of each rat.

For histomorphometry, we used a transmitted light microscope (Nikon). Image capture, processing and histomorphometry analysis was directly done using Image Pro Plus analyzer 7 software a computerized histomorphometric system (Media Cybernetics, Inc., USA). Measurements were performed using an ocular micrometer, at a magnification of 40 or 100×. The measured parameters included trabecular bone volume (TBV; expressed as a percentage of total bone volume, and obtained by dividing the trabecular area by the tissue area), bone marrow adipocyte volume (%MAV; expressed as a percentage of total bone volume, and obtained by dividing the bone marrow area by the tissue area), trabecular width, cortical thickness, and growth plate width [Ornoy and Katzburg, 1995; Somjen et al., 2006].

HORMONAL ASSAYS

LH and FSH concentration in rat serum were measured by radioimmunoassay, using the kits provided by the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Program [Yahalom et al., 2000].

CK PREPARATION AND ASSAY

Organs were collected and homogenized in buffer and centrifuged to collect extracts for creatine kinase (CK) assay as described previously [Somjen et al., 2006].

STATISTICAL ANALYSIS

Results are given as mean ± SEM. Statistical analysis was carried out using the Stat-2 software. The data were subjected to one-way analysis of variance (Kruskal–Wallis test) and Dunn's test for difference between groups.

RESULTS

MODULATION OF FSH AND LH LEVELS

As expected, FSH and LH Levels were hardly detectable in control female rats. Ovariectomy of female rats resulted in a marked increasing FSH and LH serum levels (Fig. 1a,b). OVX rats treated with E_2 had increased levels of FSH and LH and reached intermediate levels, whereas treatment of OVX rats with decapeptyl resulted in a complete suppression of serum FSH and LH levels. Treatment of OVX rats with a combination of decapeptyl + E_2, resulted in suppressed levels of FSH and LH (Fig. 1a,b).

The effects of treatments with decapeptyl and E_2 or both on bone histomorphological parameters are summarized in Table I.

Histomorphometric examination revealed a normal appearance of the intact bone; the growth plate contained the typical arrangement of cartilage cells, including proliferative, chondroblastic, and hypertrophic chondrocytes. Numerous trabecular spicules were observed underneath and adjacent to the lower aspect of the growth plate (Figs. 3a and 4a) and normal appearance of the cortex (Fig. 7a). % TBV (Fig. 5), %MAV (Fig. 6), and cortical thickness measurements (Fig. 7a,b) were within the normal range (Table I).

Vehicle-treated OVX rats (controls) were markedly osteopenic relative to control (vehicle-treated) non-OVX rats, having lost 76% of the TBV ($P < 0.01$) and 17.4% of the cortical thickness ($P < 0.001$) (Table I and Figs. 3, 5, and 7).

Ovariectomy resulted in significantly reduced growth plates, disrupted architecture (Figs. 3b and 4b). Heavy infiltration of

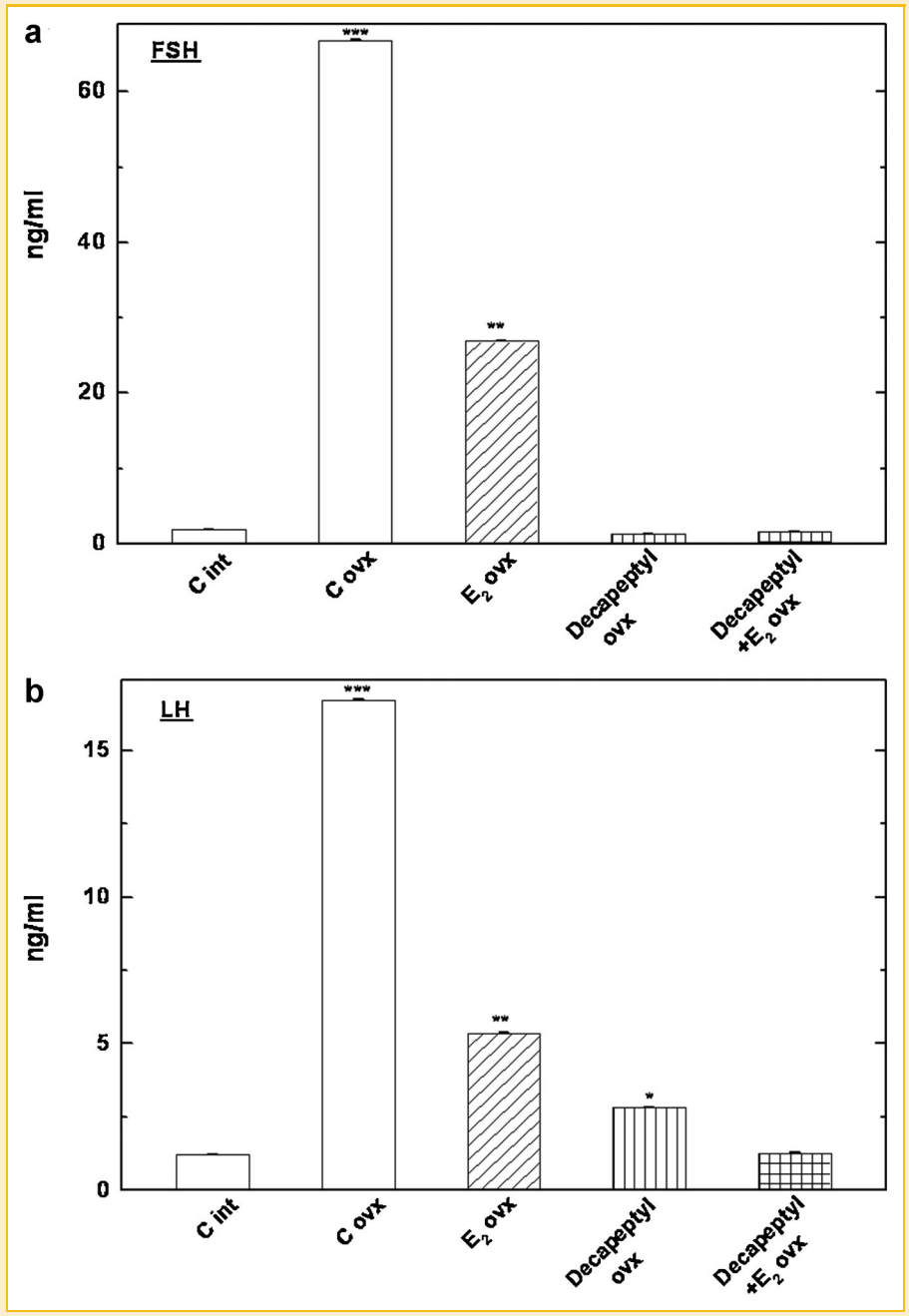

Fig. 1. Serum levels of (a) FSH and (b) LH (ng/ml) in control (C) or ovariectomized (OVX) rats treated with vehicle, or rats treated with estradiol-17β (E_2), GnRH analog + estradiol-17β (decapeptyl + E_2) as described in the Materials and Methods Section. $^{*}P < 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ from the corresponding mean values in intact control rats to the different treatments.

adipocytes into bone marrow was observed, with significant increase in %MAV (Fig. 5 and Table I), and reduction of cortical width (Fig. 7a,b and Table I). All histomorphometric parameters that were studied were significantly reduced except for the trabecular bone thickness value (Fig. 4a,b and Table I).

THE EFFECTS OF TREATMENT WITH 17β-ESTRADIOL (E_2) ON BONE

In OVX rats, treatment with E_2 completely restored the OVX-induced reduction of the features. The tibial morphological appearance was similar to that observed in intact rat bone (Fig. 4a), except for the cortical width which remained similar to that observed in non-treated OVX rats (Fig. 7a). The growth plate width was restored, and the arrangement was typical and normal containing the typical arrangement of proliferative, chondroblastic, and hypertrophic cellular populations (Fig. 3a). Thin elongated bony spicules appeared at the lower aspect of the growth plate, indicating a more synchronized growth of the bone as compared to the bonny spicules of non-treated OVX rats (Fig. 4a). E_2 amended

TABLE I. Effects of E_2 or DECA and DECA + E_2 on Bone Histological Parameters

	Percentage of trabecular bone volume (% TBV)	Trabecular thickness (spicules) (μm)	Cortical thickness (μm)	Growth plate width (μm)	Marrow Adipocyte Volume (%MAV)
Control intact	54.2 ± 4.2	44.5 ± 1.5	567.54 ± 5.0	161.0 ± 3.7	1.0 ± 0.2
OVX Control	$12.5 \pm 1.3^{@@}$	41.8 ± 1.5	$468.6 \pm 7.9^{@@}$	$101.1 \pm 4.2^{@@}$	$14.2 \pm 0.8^{@@}$
OVX E_2	$44.0 \pm 3.1^{**,@@}$	47.8 ± 1.9	$474.6 \pm 5.5^{@@}$	$136.7 \pm 3.9^{**,@}$	$4.0 \pm 0.5^{**}$
OVX DECA	$24.7 \pm 2.0^{**,@@}$	46.8 ± 2.1	$481.3 \pm 8.5^{@@}$	$94.1 \pm 2.6^{@@}$	$17.0 \pm 1.5^{@@}$
OVX DECA+E_2	$28.3 \pm 1.8^{**,@@}$	42.2 ± 1.1	$483.2 \pm 6.8^{@@}$	$109.8 \pm 3.4^{@@}$	$17.5 \pm 1.6^{@@}$

The effects of E_2 or decapeptyl or decapeptyl + E_2 on bone histomorphometry in proximal tibial growth plate and metaphysis of intact and OVX rats. Rats were injected with the different compounds as described in the Materials and Methods Section.
$^{*}P < 0.05$ from the corresponding mean values in OVX control rats.
$^{**}P < 0.01$ from the corresponding mean values in OVX control rats.
$^{b}P < 0.05$ from the corresponding mean values in intact control rats.
$^{@@}P < 0.01$ from the corresponding mean values in intact control rats.

the % TBV state to that observed in non-OVX control rats (Fig. 5 and Table I). No adipocytes were observed in this region (Fig. 6 and Table I).

THE EFFECTS OF TREATMENT WITH GnRH ANALOG (DECAPEPTYL) ON BONE

Treatment of OVX rats with decapeptyl did not improve much of the tibial morphological appearance. As seen in Figure 3a, there was a disrupted organization of the growth plate and trabecules similar to the typical of non-treated OVX state (Fig. 4a), with fewer proliferative and chondroblastic cells, and a large adipocytes population in the bone marrow with high %MAV (Fig. 6). An outstanding morphometric feature conferred by decapeptyl compared to non-treated OVX rats was a significant by 2-fold increase in TBV (Fig. 5 and Table I).

THE EFFECTS OF COMBINED TREATMENT WITH DECAPEPTYL + E_2 ON BONE

All bone parameter measures after treatment of OVX rats with combined decapeptyl + E_2, showed an intermediate state between non-treated OVX rats and intact rats (Table I). The growth plate architecture seemed partially restored but its width remained unchanged with more proliferative chondrocytes and less hypochondroblasts (Fig. 3a). Although %TBV was significantly improved, it did not reach the values observed in OVX rats after treatment with E_2 alone (Fig. 5). The other parameters were not

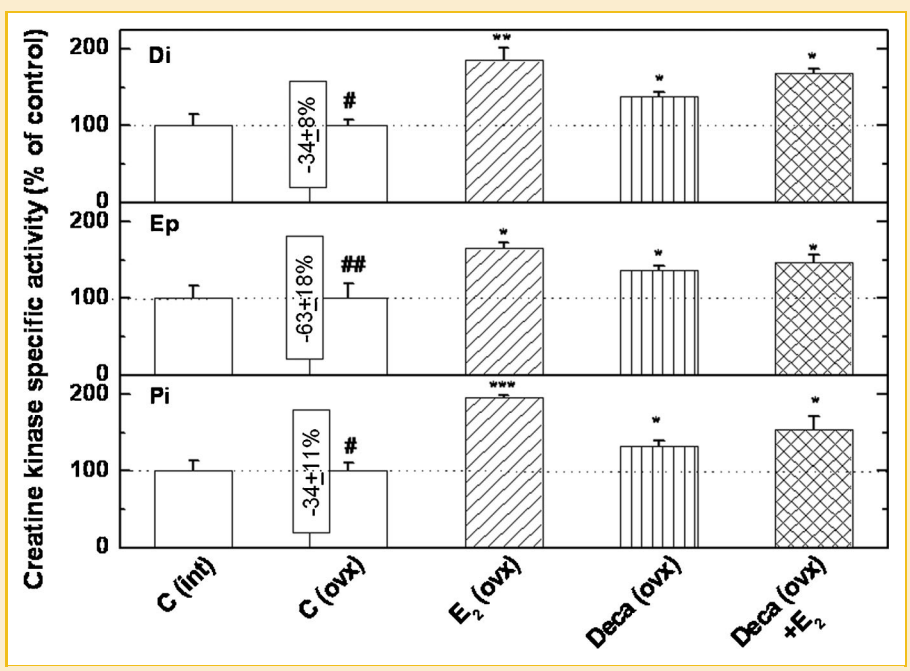

Fig. 2. The effect of E_2, decapeptyl, and decapeptyl + E_2 injected as described in experimental procedures on CK in epiphyseal cartilage (a, Ep), diaphyseal bone (b, Di), and pituitary (c, Pi) from ovariectomized female rats (Ovx). Rats were injected with E_2, decapeptyl and decapeptyl + E_2 as described in the Materials and Methods Section. Results are expressed as % change between the specific activities of CK in hormone-treated and saline injected control Ovx animals. The levels of the basal CK activity in OVX control is presented in μmol/min/mg protein for each organ. $^{\#}P < 0.05$ and $^{\#\#}P < 0.1$ for the difference between control intact and control OVX rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ from the corresponding mean values in OVX control rats to the different treatments.

improved in comparison to the non-treated OVX rat group (Table I and Figs. 3b, 4b, 6, and 7a,b).

THE EFFECTS OF DIFFERENT HORMONAL TREATMENTS ON MODULATION OF CK IN RAT ORGANS

Creatine kinase specific activity (CK) which is a marker for hormonal responsiveness in the tissues containing the relevant receptors [Kaye et al., 1997] and the main enzyme involved in energy metabolism connected to different metabolic events in the tissues [Kaye et al., 1986], was lower in all organs assayed obtained from OVX rats compared to those obtained from intact female rats, but was restored by E_2 and to lesser extent by decapeptyl. Of note is the finding that the combined decapeptyl + E_2 treatment resulted in lower CK than seen after treatment with E_2 alone (Fig. 2a–c).

DISCUSSION

In the present study, we have shown that estrogen therapy reversed the damage of ovariectomy in almost all bone histomorphometric

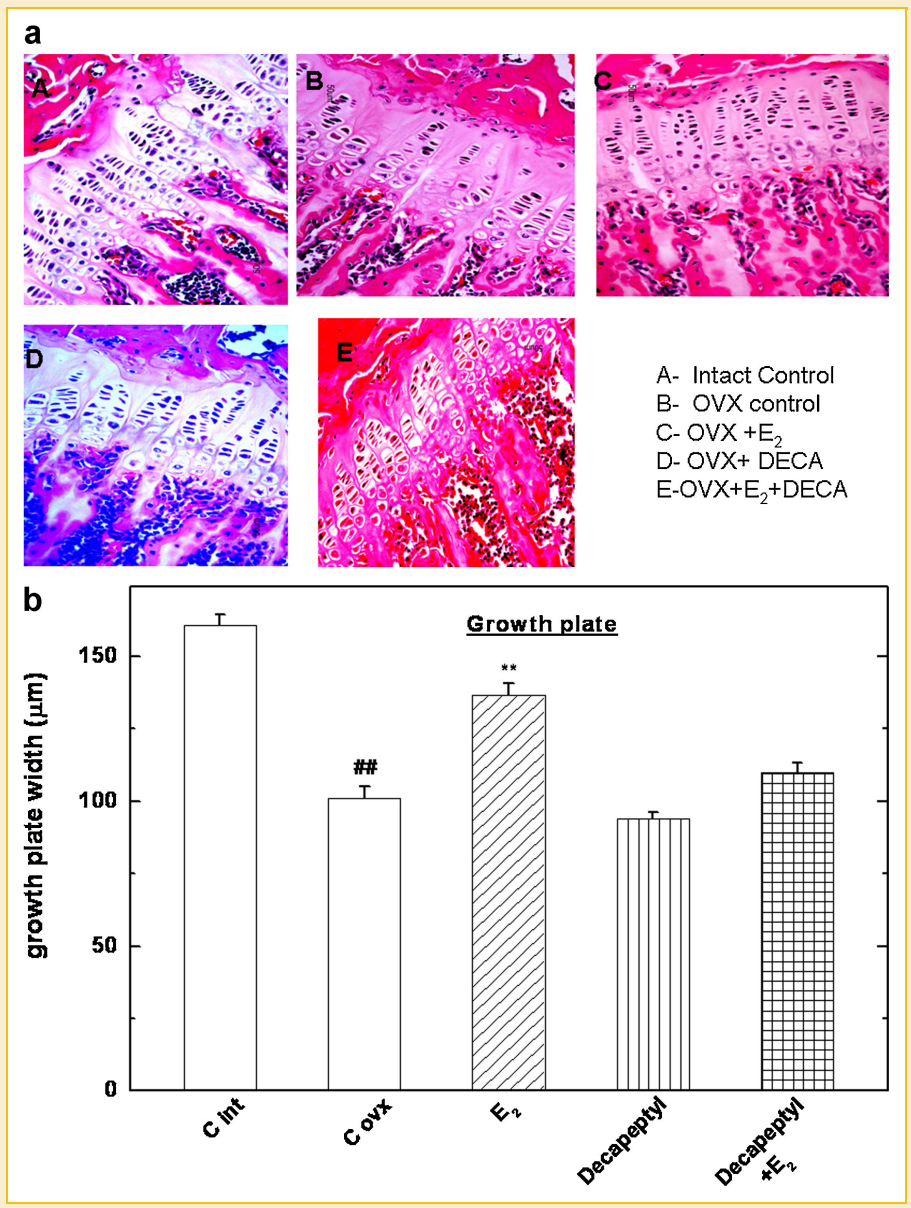

Fig. 3. a: Histological sections of female rat growth plate of the tibiae. Rats were injected with E_2, decapeptyl and decapeptyl + E_2 as described in the Materials and Methods Section. Paraffin-embedded sections were stained with hematoxylin and eosin. Magnification was 200×. b: Quantitation of the effects of E_2, decapeptyl and decapeptyl + E_2 on histomorphometry of OVX rat tibial growth plate. Rats were injected with these compounds as described in the Materials and Methods Section. $^{**}P < 0.01$ from the corresponding mean values in OVX control rats. $^{\#\#}P < 0.01$ from the corresponding mean values in intact control rats. (A scale bar is shown for 500 μm as in legend to Fig. 4a). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

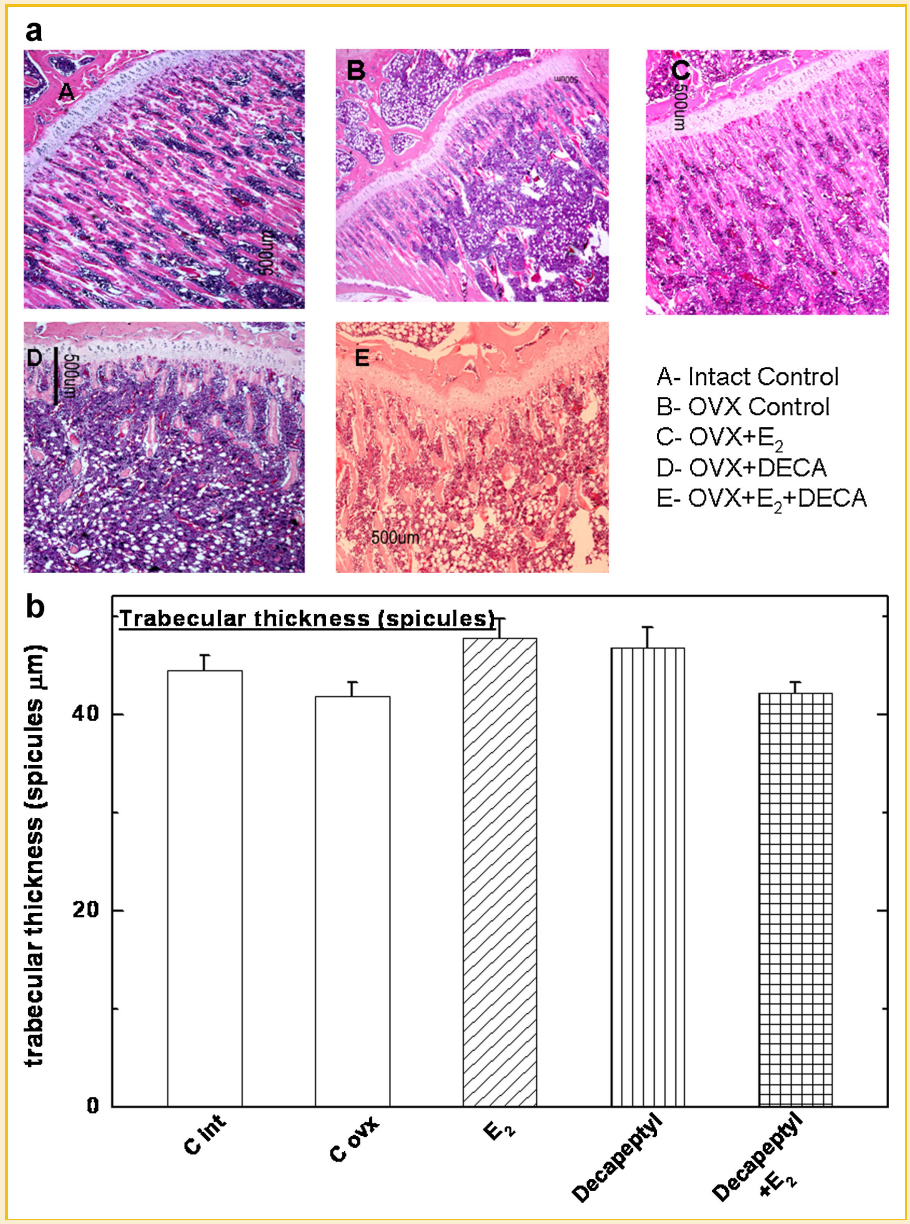

Fig. 4. a: Histological sections of female rat tibial growth plate. Rats were injected with E_2, decapeptyl, and decapeptyl + E_2 as described in the Materials and Methods Section. Paraffin-embedded sections were stained with hematoxylin and eosin. Magnification was 100×. b: Quantitation of the effects of E_2, decapeptyl, and decapeptyl + E_2 on histomorphometry of OVX rat tibial trabecular thickness. Rats were injected with the several compounds as described in the Materials and Methods Section. $^{**}P < 0.01$ from the corresponding mean values in OVX control rats. $^{\#\#}P < 0.01$ from the corresponding mean values in intact control rats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

parameters, whereas lowering the level of gonadotropins, that is, FSH and LH levels by the use of decapeptyl improved TBV only. Treatment with decapeptyl enhanced the maturation of the tibiae, reflecting a mature stage of bone observed in aged rats [Kalu et al., 1989; Schirman-Hildesheim et al., 2005]. A cluttered growth plate was observed and the zone of resorption was disarranged with thick trabeculare bone spicules. Combined E_2 + decapeptyl treatment improved trabecular volume significantly but partially restored growth plate architecture and width, and failed to affect other parameters. Hence, the addition of decapeptyl to E_2 replacement had detrimental effects. In other words, lowering high post-menopausal FSH levels to an intermediate level (here by estrogen supplementation) improved bone histomorphometry, but total suppression of FSH release to undetectable levels through the administration of a long-acting GnRH analog had no additional benefit, except for improvement in TBV. These results suggest that estrogen deficiency is the dominant factor impairing bone in the OVX state, whereas decapeptyl seems to have a selective effect only.

Studies by Sun et al. [2006] examined FSH for several reasons. In post-menopausal women, levels of FSH correlate with markers of bone resorption. Moreover, mice with deletion of both estrogen receptors α and β experience only mild bone loss. Perhaps most

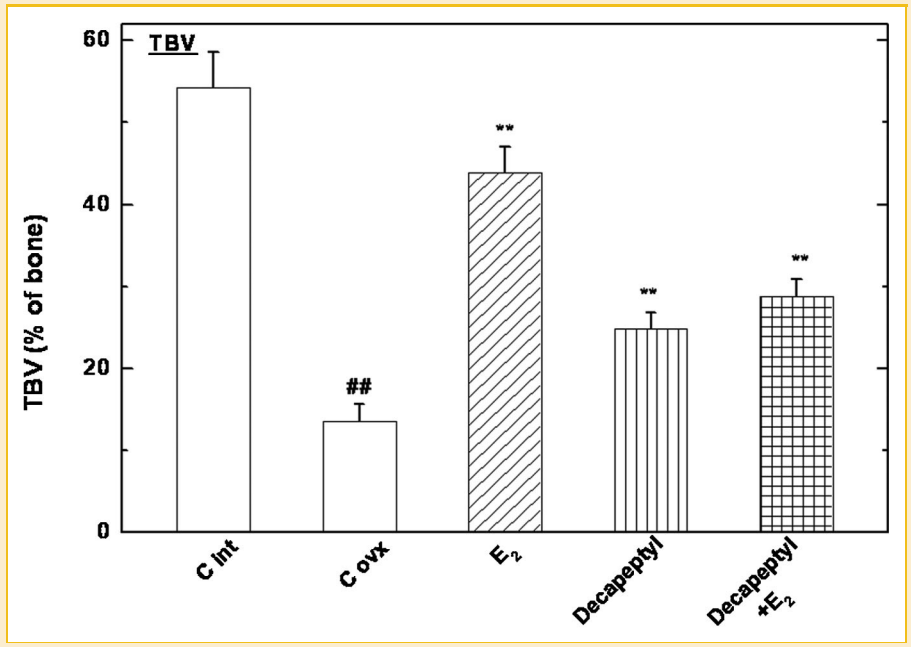

Fig. 5. Quantitation of the effects of E_2, decapeptyl, and decapeptyl + E_2 on %TBV in proximal tibial metaphysis of intact and Ovx rats. Rats were injected with the different compounds as described in the Materials and Methods Section. $^{**}P < 0.01$ from the corresponding mean values in OVX control rats. $^{\#\#}P < 0.01$ from the corresponding mean values in intact control rats.

importantly, removing the ovaries in mice causes severe bone loss, but much of this bone loss apparently depends on the presence of an intact pituitary [Huang et al., 2001; Shimasaki et al., 2004]. Their data suggested that FSH, acting via its cognate receptor, was capable of regulating osteoclastogenesis and bone resorption, independent of estrogen levels, and they convincingly established the presence of FSHR on osteoclasts and their direct responsiveness to FSH. Yet, the conclusion that hypogonadal bone loss is caused by FSH, independent of estrogen might be simplistic, since our own model merely reproduced already well-established observations that

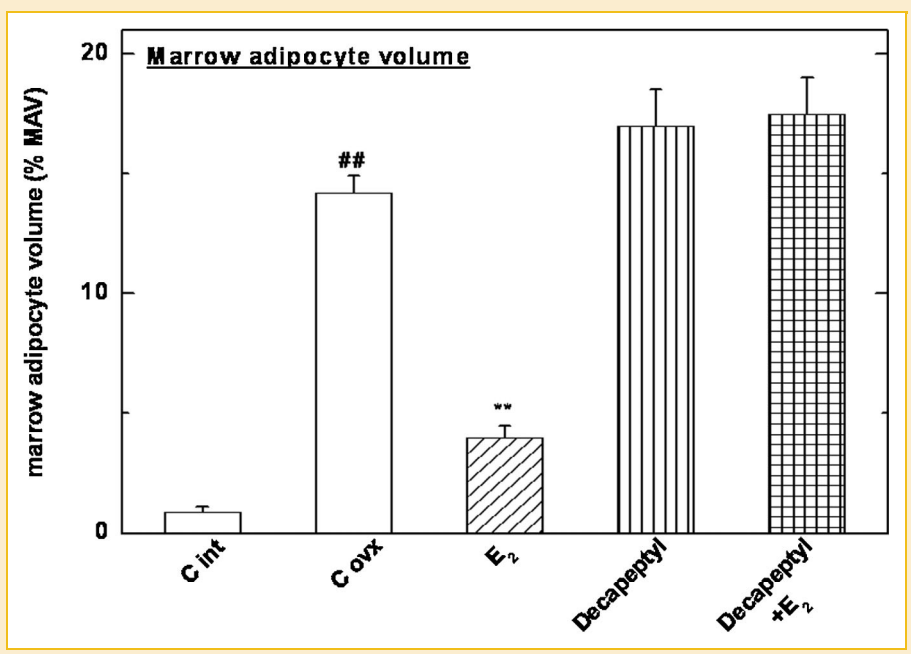

Fig. 6. Quantitation of the effects of E_2, decapeptyl, and decapeptyl + E_2 on histomorphometry of OVX rat %MAV. Rats were injected with the E_2, decapeptyl, and decapeptyl + E_2 as described in the Materials and Methods Section. $^{**}P < 0.01$ from the corresponding mean values in OVX control rats. $^{\#\#}P < 0.01$ from the corresponding mean values in intact control rats.

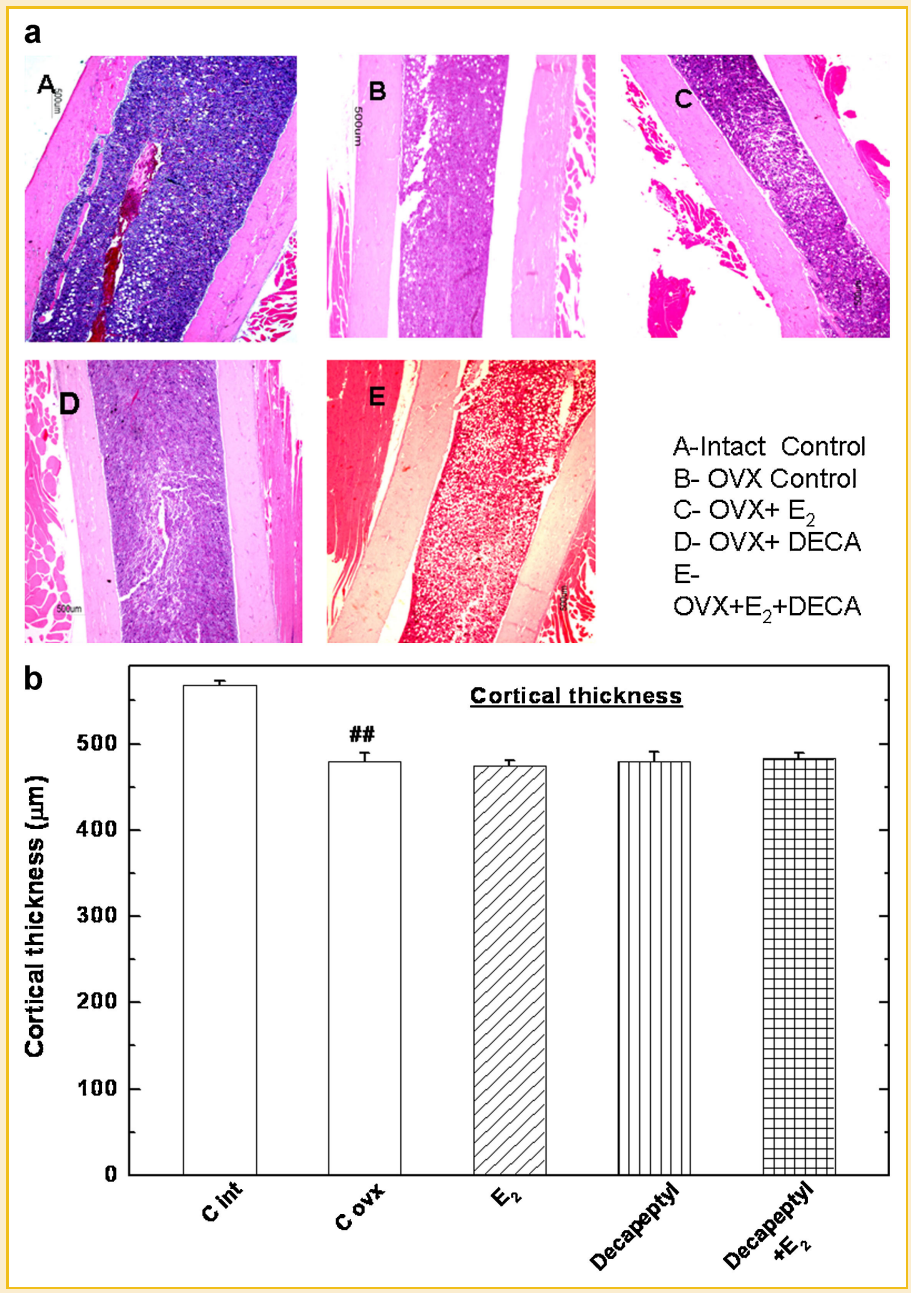

Fig. 7. a: Histological sections of female rat cortex. Rats were injected with E_2, decapeptyl, and decapeptyl + E_2 as described in the Materials and Methods Section. Paraffin-embedded sections were stained with hematoxylin and eosin. Magnification 20×. b: Quantitation of the effects of E_2, decapeptyl, and decapeptyl + E_2 on histomorphometry of OVX rat tibial cortical thickness. Rats were injected with these compounds as described in the Materials and Methods Section. $^{**}P < 0.01$ from the corresponding mean values in OVX control rats. $^{\#\#}P < 0.01$ from the corresponding mean values in intact control rats. (A scale bar is shown for 500 μm as in legend to Fig. 4a.) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

simple correcting estrogen deficiency is sufficient to treat low bone mass in animal models as well as in patients.

Consistent with this concept, observations in estrogen receptor (ER) knockout mice provide suggest that sex hormones alone can regulate bone mass independent of FSH: double ER null female mice have a low bone mass [Yeh et al., 1997; Sims et al., 2002], despite normal levels of FSH [Couse and Korach, 1999; Couse et al., 2003]. Furthermore, the surprisingly normal bone mass in ERα null mice does not indicate irrelevance of ERα in this setting; rather, in the absence of ERα, circulating levels of androgen are markedly increased in both males and females [Couse and Korach, 1999], which prevents bone loss via the androgen receptor [Sims et al., 2003]. To further clarify this issue, studies by Gao et al. [2007] showed the influence of ovarian transplantation, ovariectomy, androgen receptor blockade and aromatase inhibition in the FORKO mice and showed that skeletal maintenance is ovary-dependent in

this FSHR-deficient mouse. It was also that ''activating'' polymorphism of FSHR causes low bone mass [Rendina et al., 2010]. Also elevated FSH contributes to bone loss across the menopausal transition [Iqbal et al., 2010], and the FSH inhibitor leuproperlin has protective effects on bone loss [Liu et al., 2010]. There is also information that FSH does not regulate bone resorption in postmenopausal women [Drake et al., 2010]. Similarly we show here that estrogen, rather than gonadotropins, affects bone histomorphometry in the OVX state.

These studies therefore demonstrate a critical role for estrogen and androgen in the maintenance of bone mass, despite unchanged FSH levels. LH levels, which were not reported in studies by Sun et al. [2006, 2008] may have induced an increase in serum androgen levels. In our study, the use of the GnRH analog led to suppression of FSH and LH levels, thereby allowing us to establish clearly that low estrogen rather than high FSH/LH levels is responsible for bone loss. However, concomitant changes in the serum levels of gonadotropic hormones achieved by decapeptyl do have some complex and selective modulating role in this setting.

Whereas FSH may have some direct effects on bone, it is not the only pituitary hormone that has been investigated in this setting [Imam et al., 2009]. Oxytocin has anabolic effects on bone was shown to direct differentiation of human mesenchymal stem cells towards osteogenesis [Bassett and Williams, 2008; Elabd et al., 2008; Tamma et al., 2009]. It was proposed a direct and important role for thyrotropin in skeletal development as a key negative regulator of bone turnover [Abe et al., 2007; Sun et al., 2008; Liu et al., 2009; Zaidi et al., 2009], whereas in studies by Bassett et al. [2008] it was claimed that thyroid hormones rather than thyrotropin regulate skeletal development in models of hypothyroidism. Recently, studies by Otani et al. [2009] showed that hypothalamic GT1-7 neuron cells express functional machinery of the bone morphogenetic proteins (BMPs) system. BMPs which belong to the family of transforming growth factor-β, were originally identified as the active components of bone extracts capable of inducing bone formation at ectopic sites. Recent studies have shown that BMPs play crucial roles in female reproduction not only by regulating ovarian steroidogenesis and mitogenesis [Otsuka and Shimasaki, 2002b; Shimasaki et al., 2004] but also by activating pituitary gonadotropins secretion in an autocrine/paracrine manner [Huang et al., 2001; Otsuka and Shimasaki, 2002a,b; Nicol et al., 2008]. However, the effects of BMPs on hypothalamic GnRH production and secretion have yet to be elucidated. Studies by Otani et al. [2009] unrevealed a novel interaction between BMPs and ER, which is involved in controlling GnRH production and secretion.

One of the outstanding features conferred by decapeptyl treatment was a large adipocyte population with a high %MAV similar to that in the OVX state, which was not improved by estrogen supplement as was observed in the OVX rats treated with combined decapeptyle with estrogen. Mesenchymal stem cells from bone marrow stroma are capable of differentiating into osteoblasts and adipocytes, among other cell phenotypes [Rodríguez et al., 2008]. The protective effects exerted by locally generated factors such as estradiol-17β is established [Martin and Gaddy, 2006; Gao et al., 2007] and as already mentioned oxytocin differentiation of human mesenchymal stem cells towards osteogenesis. Identifying signaling pathways that stimulate mesenchymal cells osteogenesis is of major importance for better understanding osteoporosis, and developing new therapeutic options. In this setting, the effect on GnRH on mesenchymal stem cells fate should be investigated.

There is a body of evidence for the existence of complex interactions between hypothalamus, pituitary, gonads and bone, and the possibility of a direct hypothalamic–pituitary–bone axis has been suggested [Bassett and Williams, 2008; Imam et al., 2009]. Nevertheless, despite the emerging concept that bone can be directly regulated by the hypothalamic–pituitary–gonadal axis, the paradigm of FSH causing hypogonadal bone loss cannot yet replace the traditional estrogen-deficiency-osteoporosis paradigm.

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