Journal of Steroid Biochemistry & Molecular Biology 125 (2011) 226–230

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Sex specific response of cultured human bone cells to ERα and ERβ specific agonists by modulation of cell proliferation and creatine kinase specific activity

Dalia Somjen[a,*], Sara Katzburg[a], Orli Sharon[a], Esther Knoll[a], David Hendel[b], Naftali Stern[a]

[a] Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 64239, Israel
[b] Sharei-Zedek Medical Center, Jerusalem, Israel

ARTICLE INFO

Article history:
Received 6 December 2010
Received in revised form 2 March 2011
Accepted 4 March 2011

Keywords:
DPN
PPT
Osteoblasts
Lipoxygenase
HETE
DNA
CK

ABSTRACT

We have previously reported that human cultured bone cells (hObs) respond to estradiol-17β (E2) by stimulating DNA synthesis, creatine kinase BB specific activity (CK) and other parameters sex-specifically. We now investigate the sex specificity of the response of these hObs to estrogen receptor (ER) α and ERβ specific agonists. Real time PCR revealed that all cells express mRNA for both ERs. ERα mRNA but not ERβ mRNA was stimulated by all estrogenic compounds in both pre- and post-menopausal hObs with no effect in male hObs. Cells treated with E2, 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN; ERβ specific agonist) and 4,4′,4″-[4-propyl-(1H)-pyrazol-1,3,5-triyl] tris–phenol (PPT; ERα specific agonist) showed increased DNA synthesis and CK in all female but not male hObs. Raloxifene (Ral), a specific ERα antagonist, inhibited the stimulation of DNA synthesis and CK by E2 or PPT, but not by DPN. DPN and PPT like E2 modulated the expression of both 12 and 15 lipooxygenase (LO) mRNA in both female but not male hObs. 12 and 15 HETE production was modulated only by DPN and PPT in these cells. The LO inhibitor baicaleine inhibited only E2 and PPT but not DPN effects in both female hObs. In conclusion, we provide herein evidence for the separation of age- and sex-dependent mediation via both ERα and ERβ pathways in the effects of estrogens on hObs, with a yet unknown mechanism.

1. Introduction

Estrogens appear to confer multiple skeletal protective effects *in vitro* [1–4] and *in vivo* [5–7], and to positively affect skeleton outcome in prospective trials in post-menopausal women [8,9]. We found that vitamin D, PTH as well as other hormones including phytoestrogens stimulate DNA and CK as well as LO mRNA and HETE formation [10,11]. We also found that the ERα and ERβ specific agonists have differential effects on all these parameters in the human osteoblastic cell line $SaSO_2$ [12]. In the present study we analyzed the response of primary human osteoblasts in culture from males or females to the ERα agonist PPT and the ERβ agonist DPN at different parameters, in order to see whether the sex-specificity is retained also using the specific ERs agonist similar to E2 itself.

We analyzed specifically the expression and regulation of lipoxygenase (LO) enzymes in these human bone cells. We focused on these enzymes in osteoblasts since LO products were shown to modulate MAPK activity [13,14] and proliferation or survival [15] in a number of cell types and could thus play a role in promoting cell growth, either independently or through estrogens action. Additionally, there is recent strong circumstantial evidence linking LO expression to bone mineral content [15,16]. We hypothesized that the growth promoting effects of estrogens in human osteoblast-like cells may be associated with accelerated production of LO metabolites, whose putative action may explain some of the newly found links between LO and bone density.

We now attempt to determine whether or not the estrogens receptor specific multiple ligands affect primary cultures of osteoblast-like cells (hObs) derived from pre- or post-menopausal cells compared to male-bone derived cells and to see whether the effects are similar to what was found using $SaSO_2$ cells [12].

We set to determine: (1) Whether or not these receptor specific ligands affect ERα and ERβ mRNA expression. (2) Whether or not the receptor specific ligands affect DNA synthesis and CK specific activity in hObs with and without ERα specific inhibitor raloxifene (Ral). (3) Whether or not the receptor specific ligands affect LO mRNA expression and its activity by stimulating the production of HETEs in Obs. (4) To evaluate whether LO activity inhibitor baicaleine inhibit DNA synthesis and CK specific activity induced by the receptor specific ligands of the estrogens receptors in Obs.

We found that: (1) All compounds tested increased the expression of both ERs' mRNA in hObs from pre- but not post-menopausal or male-derived bone cells. (2) E2 as well as PPT and DPN increases

* Corresponding author at: Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Centre and the Sackler Faculty of Medicine, Tel Aviv University, Tel.:-Aviv 64239, Israel. Tel.: +972 3 697 3812; fax: +972 3 697 3421.
E-mail address: dalias@tasmc.health.gov.il (D. Somjen).

0960-0760/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2011.03.006

DNA synthesis and CK specific activity in hObs from pre- and post-menopausal but not male-derived bone cells. (3) Only E2 as well as PPT but not DPN increases in DNA synthesis and CK specific activity in hObs from pre- and post-menopausal derived bone cells were inhibited by Ral. (4) All estrogens modulated both 12- and 15 LO mRNA expression in hObs from pre- and post-menopausal but not male-derived bone cells. (5) DPN and PPT stimulated the production of 12HETE in hObs from pre- and post-menopausal but not male-derived bone cells. Only PPT stimulated the production of 15HETE in hObs from pre- and post- menopausal but not male-derived bone cells. (6) Baicaleine which inhibits 12- and 15-HETE formation through blockade of 12 and 15 LO activity, inhibited DNA synthesis and CK specific activity stimulation by E2 and PPT but not by DPN.

In conclusion, our data suggest that E2 as well as PPT (ERα specific) and DPN (ERβ specific) increases DNA synthesis and CK specific activity in hObs from pre- and post-menopausal but not male-derived bone cells, in part via induction of lipoxygenase enzymes in a yet unclear mechanism (s). This feature of estrogenic compounds appears to be shared by multiple members of the estrogens family and is not exclusively linked to either ERα or ERβ as well as membranal binding sites, but is sex specific.

2. Materials and methods

Creatine kinase assay kit was from Sigma Chemicals Co. (St. Louis, MO). DPN and PPT were from Tocris biosciences (Bristol, BS11 OQI, UK). Raloxifene (marketed as Evista) was from Eli Lilly and Company. All other reagents used were of analytical grade.

2.1. Cell cultures

Primary human bone cells (hObs) from pre- and post-menopausal females or males were prepared by us [17].

2.2. Hormonal treatment

Sub-confluent cells were treated daily with vehicle, E2 at 10 nM or DPN at 42 nM and PPT at 39 nM;

(a) for 3 days daily for the expression of mRNA for 12LO and 15LO type 1 expression or for estrogen receptor α (ERα) and estrogen receptor β (ERβ) were determined as previously described [18,19] or;
(b) for 1 h with serum-free medium, followed by the addition of vehicle or estrogenic compounds: E2 at 30 nM, DPN at 420 nM and PPT at 390 nM for 10 min and HETE were extracted and assayed as previously described [11,12,20] or;
(c) for 24 h with the addition of vehicle or estrogenic compounds: E2 at 30 nM, DPN at 420 nM and PPT at 390 nM for DNA synthesis and for CK specific activity as previously described [12,20]. In these experiments raloxifene (Ral) was used at 3000 nM.

2.3. Determination of mRNA by RT-PCR

2.3.1. For ERα and ERβ

RNA was extracted and expression of ERα and ERβ was carried out by RT-PCR as described previously [18,19].

2.3.2. For 12 and 15LO by RT-PCR

RNA was extracted and expression of 12 and 15LO enzymes was carried out by RT-PCR as described previously [11,12,20].

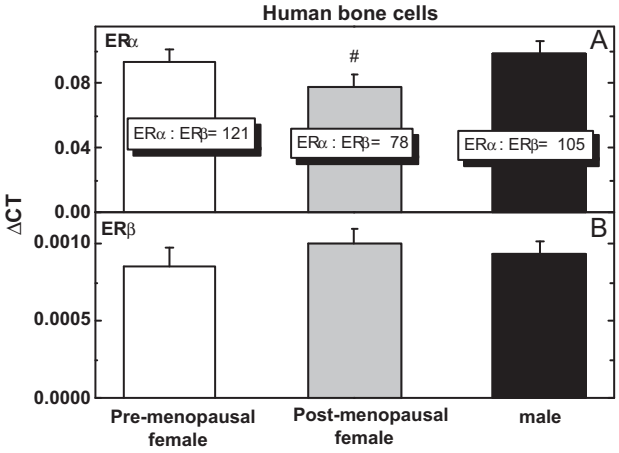

Fig. 1. The expression of ERα and ERβ mRNA in cultured human female and male bone cells and the ratio of the expression of ERα to ERβ. Cells were obtained, cultured and analyzed as described. Number of cultured bone specimen $n = 5$ for each group, $\#p < 0.05$.

2.4. Determination of the levels of 12 and 15HETE by HPLC

Cells and medium were extracted for HETE and analyzed by HPLC as described previously [11,12,20].

2.5. Assessment of DNA synthesis and creatine kinase extraction and assay

Twenty-two hours after hormonal treatment, ^{3}[H] thymidine was added for 2 h and its incorporation into DNA was determined as described previously [11,12,20] or cells were scraped off the culture dishes and homogenized by freezing and thawing three times in an extraction buffer as previously described [11,12,20]. Supernatant extracts were obtained by centrifugation of homogenates at 14,000 × g for 5 min at 4 °C in an Eppendorf micro centrifuge. Creatine kinase activity (CK) was assayed by a coupled spectrophotometric assay described previously [11,12,20]. Protein was determined by Coomasie blue dye binding using bovine serum albumin (BSA) as the standard.

2.6. Statistical analysis

The significance of differences between experimental and control means was evaluated using Student's t-test or ANOVA, in which $n = 5$–8 number of cultures. $p > 0.05$, was considered significant.

3. Results

3.1. Modulation of ERα and ERβ mRNA expression in cultured human female and male bone cells by estrogenic compounds

Both ERα (Fig. 1A) and ERβ (Fig. 1B) mRNA are expressed in cultured human pre- or post-menopausal female or male bone cells (hObs) with higher abundance of ERα in all hObs (Fig. 1). Three daily additions of E2 (10 nM), DPN (42 nM) or PPT (39 nM) to bone cells, modulated the expression of mRNA for ERβ (Fig. 2B) and ERα in hObs (Fig. 2A). All hormones stimulated ERα and ERβ expression in pre-menopausal female hObs, but only E2 and PPT modulated the expression of mRNA for ERα in post-menopausal female hObs (Fig. 2A). Only DPN decreased the expression of mRNA for ERβ in post-menopausal female hObs (Fig. 2B). None of the hormones affected the expression of any of the ERs mRNA expression in male derived bone cells (Fig. 2).

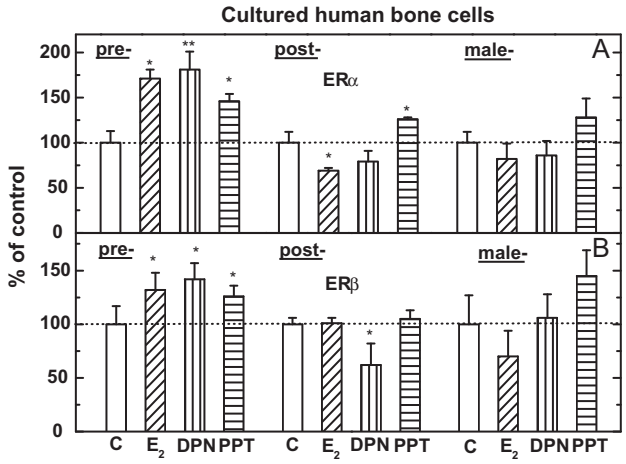

Fig. 2. The effect of E2, DPN and PPT on the expression of ERα and ERβ mRNA in cultured pre- or post-menopausal female and male human bone cells. Cells were obtained, cultured as well as treated by the different hormones and analyzed as described. Number of cultured bone specimen $n = 5$ for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

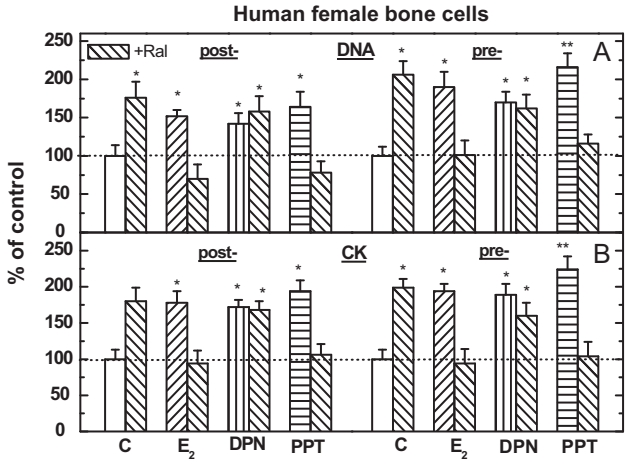

Fig. 4. The effect of E2, DPN and PPT in the presence of raloxifene (Ral) on DNA synthesis and on CK specific activity in pre- or post-menopausal cultured human female bone cells. Cells were obtained, cultured as well as treated by the different hormones and analyzed as described. Number of cultured bone specimen $n = 5$–10 for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

3.2. The effect of estrogenic compounds on DNA synthesis and CK specific activity in cultured human female and male bone cells

Addition of E2 (30 nM), DPN (420 nM) or PPT (390 nM) to bone cells, induced DNA synthesis (Fig. 3A) and CK specific activity (Fig. 3B) in both female derived hObs, but not in male derived Obs.

3.3. The effect of estrogenic compounds on DNA synthesis and CK specific activity in cultured human female bone cells in the presence of raloxifene

Addition of raloxifene (Ral at 3000 nM) to E2 (30 nM), DPN (420 nM) or PPT (390 nM) to both female derived hObs modulated the induction of DNA synthesis (Fig. 4A) and CK specific activity (Fig. 4B). Ral inhibited the stimulations by E2 and PPT but did not affect the stimulatory effects of DPN (Fig. 4).

3.4. Modulation of LO mRNA expression in cultured human female and male bone cells by estrogenic compounds

Three daily additions of E2 (10 nM), DPN (42 nM) or PPT (39 nM) to bone cells, modulated the expression of mRNA for 12LO (Fig. 5A) and 15LO (Fig. 5B) in female and male hObs. All compounds stimulated 15LO mRNA expression in pre- and post-menopausal female hObs (Fig. 5B). On the other hand E2 and DPN stimulated 12LO mRNA expression with only PPT inhibiting it in these cells (Fig. 5A). E2 also decreased the expression of mRNA for 12LO in male hObs (Fig. 5A).

3.5. Modulation of HETE formation in cultured human female and male bone cells by estrogenic compounds

Additions of E2 (30 nM), DPN (420 nM) or PPT (390 nM) to bone cells, modulated the formation of 12 and 15HETE in hObs (Fig. 6). DPN and PPT stimulated 12HETE formation in pre-and post-

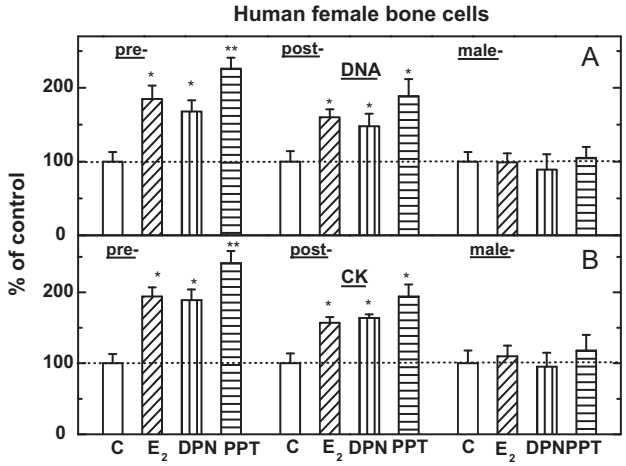

Fig. 3. The effect of E2, DPN and PPT on DNA synthesis and on CK specific activity in pre- or post-menopausal female and male cultured human bone cells. Cells were obtained, cultured as well as treated by the different hormones and analyzed as described. Number of cultured bone specimen $n = 5$–10 for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

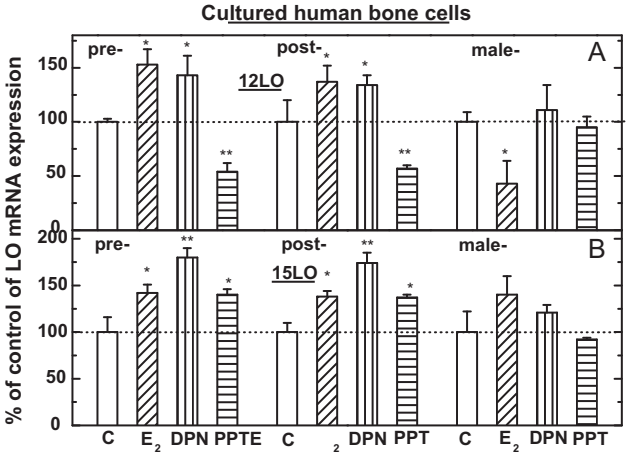

Fig. 5. The effects of E2, DPN and PPT on the expression of 12LO and 15LO mRNA in cultured pre- or post-menopausal female and male human bone cells. Cells were obtained, cultured as well as treated by the different hormones and analyzed as described. Number of cultured bone specimen $n = 5$ for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

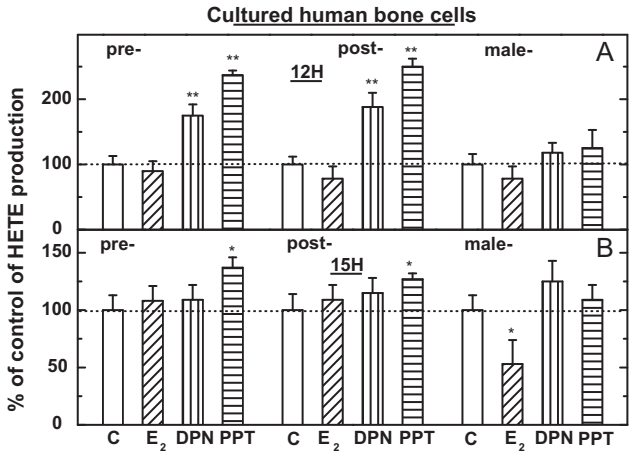

Fig. 6. The effects of E2, DPN and PPT on the formation of 12HETE and 15HETE in cultured pre- or post-menopausal female and male human bone cells. Cells were obtained, cultured as well as treated by the different hormones and analyzed as described. Number of cultured bone specimen $n = 5$ for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

menopausal female hObs (Fig. 6A) and only PPT induced 15HETE in these cells (Fig. 6B). Only E2 inhibited 15HETE formation in male hObs (Fig. 6B).

3.6. The effect of estrogenic compounds on DNA synthesis and on CK specific activity in cultured human female bone cells with and without baicaleine

Treatment of pre- or post-menopausal female hObs for 24 h with estrogenic compounds: E2 (30 nM), DPN (420 nM) or PPT (390 nM) showed increased DNA synthesis and CK specific activity (Fig. 7). Baicaleine (baic, 1000 nM) the inhibitor of 12 and 15HETE synthesis, abolished the E2- and PPT- but not DPN-stimulation of DNA synthesis and not CK specific activity in both female hObs (Fig. 7).

4. Discussion

The effects of estrogenic compounds both the ERα specific agonist PPT, the ERβ specific agonist DPN as well as E2 on primary human derived cultured hObs complements our findings in

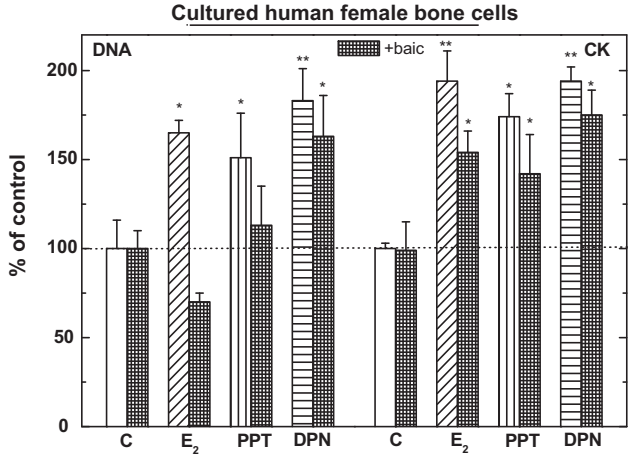

Fig. 7. The effect of E2, DPN and PPT with and without the HETE formation inhibitor baicalein (baic) on DNA synthesis and on CK specific activity in pre- and post-menopausal cultured human female bone cells. Cells were obtained, cultured as well as treated by the different hormones and analyzed as described. Number of cultured bone specimen $n = 5$–10 for each group, $^{*}p < 0.05$, $^{**}p < 0.01$.

human skeletal cells *in vitro*, where E2 stimulate cell proliferation, energy metabolism as well as other responses mediated by membranal binding sites [19,22] only in female- but not male-derived cells. We also demonstrated changes in the lipoxygenase systems. Although reports suggest that 12 and 15HETE interact with multiple signals promoting cell replication, their effects are probably not redundant, since inhibition of their production hinders normal cell growth in a variety of cell types [16]. Recent publications linked also 12/15LO or 15LO and 12LO (platelet type) to bone density [16,22]. In this study we provide the first direct evidence for the expression and biological role of LOs in bone cell biology. We found mRNA expression of three types of LOs in cultured human bone cells and lines, i.e., the platelet type 12LO, 15LO type 1 and 15LO type 2 [10]. Here we show that the expression of the LOs in these cells not only by modulated by vitamin D metabolites and analogs [11,20] or PTH [10], but also by the estrogenic compounds as well [21]. The expression of these enzymes results in the ability of hObs to secrete 12HETE and 15HETE, the products of LO. Moreover, the generation of HETE which is driven by the estrogenic compounds is linked to hObs growth induced by these hormones. The HETE raise local oxidative stress as was shown by us previously [11,20] by measurements of ROS formation induced by E2, DPN and PPT. Because oxidative stress, in turn, may lead to inhibition of differentiation of bone osteoblasts-like cells [17] and acceleration of osteoclast differentiation [16], its induction may also result in the release of oxidizing fatty acids which unfavourably affect overall bone osteoblast/osteoclast homeostasis through enhanced oxidative stress. Presently, however, these potential secondary sequels of estrogens-stimulated LO activities in bone remain entirely conjectural and are the subject of future investigation. This previously unrecognized feature of estrogenic compounds appears to be shared by multiple members of the estrogens family and is not exclusively linked to either ERα or ERβ and/or membranal binding sites. The most serious potential hazard of these effects could be unwanted induction of inflammatory reaction.

We also demonstrated here similar to what we found previously [21] that there is sex specificity in the response of human derived cultured osteoblasts to ERs-specific agonists as well as to E2 itself or to different phytoestrogens and their synthetic derivatives [21]. Of interest is the fact that male-derived bone cells which did not respond to estrogenic compounds in the different parameters tested, expressed mRNA for both estrogen receptors similar to female derived bone cells and especially post-menopausal cells, suggesting post-receptor mechanism(s) in determining the ability of the cells to respond to the hormones [23,24]. It is also important to note that the "less differentiated" cells of the epiphyseal cartilage which can be considered as "pre-osteoblasts" and the "de-differentiated" bone cells derived from gonadectomized animals lost their sex-specific response to gonadal steroids.

Whether these findings imply also to human bone *in vivo* is yet to be established. Although recently it was published in a review that in human subjects *in vivo* there is also sex-specific, the role for the gonadal steroids in the quality and the response to gonadal hormones [25] and especially the origin of the lack of response of male-derived cells is not yet understood. Whether it is due to changes in their "differentiation status" and/or the lack of post-receptor factors and pathways has still to be tested.

References

[1] D. Somjen, Y. Weisman, Z. Mor, A. Harell, A.M. Kaye, Regulation of proliferation of rat cartilage and bone by sex steroid hormones, J. Steroid Biochem. Mol. Biol. 40 (1991) 717–723.
[2] A.M. Kaye, Y. Weisman, A. Harell, D. Somjen, Hormonal stimulation of bone cell proliferation, J. Steroid Biochem. Mol. Biol. 37 (1990) 431–435.

[3] B. Fournier, S. Haring, A.M. Kaye, D. Somjen, Stimulation of creatine kinase specific activity in human osteoblast and endometrial cells by estrogens and anti-estrogens and its modulation by calciotropic hormones, J. Endocrinol. 150 (1996) 275–285.
[4] S. Katzburg, A. Ornoy, D. Hendel, M. Lieberherr, A.M. Kaye, D. Somjen, Age and gender specific stimulation of creatine kinase specific activity by gonadal steroids in human bone-derived cells in culture, J. Endocrinol. Invest. 24 (2001) 166–172.
[5] D. Somjen, Y. Weisman, A. Harell, E. Berger, A.M. Kaye, Direct and sex specific stimulation by sex steroids of creatine kinase activity and DNA synthesis in rat bone, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 3361–3363.
[6] J.C. Gallagher, Drug therapy of osteoporosis: calcium, estrogen and vitamin D, in: B.L. Riggs, L.J. Melton III (Eds.), Osteoporosis: Etiology, Diagnosis and Management, vol. 1, Raven Press, New York, 1988, pp. 389–401.
[7] S.C. Manolagas, S. Kousteni, R.L. Jilka, Sex steroids and bone, Recent Progr. Horm. Res. 57 (2002) 385–409.
[8] P.D. Delmas, Treatment of postmenopausal osteoporosis, Lancet 359 (2000) 2018–2026.
[9] Effects of menopause and estrogen replacement therapy or hormone replacement therapy in women with diabetes mellitus: consensus opinion of The North American Menopause Society, Menopause 7 (2000) 87–95.
[10] D. Somjen, K. Tordjman, S. Katzburg, E. Knoll, O. Sharon, R. Limor, M. Naidich, Z. Naor, D. Hendel, N. Stern, Lipoxygenase metabolites are mediators of PTH-dependent human osteoblast growth, Bone 42 (2008) 491–497.
[11] D. Somjen, S. Katzburg, M. Grafi-Cohen, E. Knoll, O. Sharon, G.H. Posner, Vitamin D metabolites and analogs induce lipoxygenase mRNA expression and activity as well as reactive oxygen species (ROS) production in human bone cell line, J. Steroid Biochem. Mol. Biol., 123 (2011) 85–89.
[12] D. Somjen, S. Katzburg, O. Sharon, M. Grafi-Cohen, E. Knoll, N. Stern, The effects of estrogen receptors α and β specific agonists and antagonists on cell proliferation and energy metabolism in human bone cell line, J. Cell. Biochem., 112 (2011) 625–632.
[13] W.G. Tong, X.Z. Ding, T.E. Adrian, The mechanisms of lipoxygenase inhibitor-induced apoptosis in human breast cancer cells, Biochem. Biophys. Res. Commun. 296 (2002) 942–948.
[14] H.J. Kim, E.J. Chang, H.M. Kim, S.B. Lee, H.D. Kim, G. Su Kim, H.H. Kim, Antioxidant α-lipoic acid inhibits osteoclast differentiation by reducing nuclear factor-κB DNA binding and prevents in vivo bone resorption induced by receptor activator of nuclear factor-κB ligand and tumor necrosis factor-α, Free Radic. Biol. Med. 40 (2006) 1483–1493.
[15] R.F. Klein, J. Allard, Z. Avnur, T. Nikolcheva, D. Rotstein, A.S. Carlo, M. Shea, R.V. Waters, J.K. Belknap, G. Peltz, E.S. Orwoll, Regulation of bone mass in mice by the lipoxygenase gene ALOx15, Science 303 (2004) 229–232.
[16] N. Mody, F. Parhami, T.A. Sarafian, L.L. Demer, Oxidative stress modulates osteoblastic differentiation of vascular and bone cells, Free Radic. Biol. Med. 31 (2001) 509–519.
[17] S. Katzburg, M. Lieberherr, A. Ornoy, B.Y. Klein, D. Hendel, D. Somjen, Isolation and hormonal responsiveness of primary cultures of human bone-derived cells: gender and age differences, Bone 25 (1999) 667–673.
[18] D. Somjen, Y. Zaltsman, B. Gayer, T. Kulik, E. Knoll, N. Stern, L.J.W. Lu, L. Toldo, F. Kohen, 6-Carboxymethyl genistein: a novel selective oestrogen receptor modulator (SERM) with unique differential effects on the vasculature, bone and uterus, J. Endocrinol. 173 (2002) 415–427.
[19] D. Somjen, F. Kohen,.M. Lieberherr, B. Gayer, E. Schejter, S. Katzburg, R. Limor, O. Sharon, E. Knoll, G.H. Posner, A.M. Kaye, N. Stern, Membranal effects of phytoestrogens and carboxy derivatives of phytoestrogens on human vascular and bone cells: new insights based on studies with carboxy-biochanin A, J. Steroid Biochem. Mol. Biol. 93 (2005) 293–303.
[20] D. Somjen, S. Katzburg, E. Knoll, O. Sharon, G.H. Posner, N. Stern, Vitamin D analogs induce lipoxygenase mRNA expression and activity as well as reactive oxygen species (ROS) production in human bone cells, J. Steroid Biochem. Mol. Biol. 121 (2010) 265–267.
[21] D. Somjen, S. Katzburg, M. Lieberherr, D. Hendel, I. Yoles, DT56a stimulates gender-specific human cultured bone cells in vitro, J. Steroid Biochem. Mol. Biol. 98 (2006) 90–96.
[22] S. Ichikawa, D.L. Koller, M.L. Johnson, D. Lai, X. Xuei, R.F. Edenberg, E.S. Klein, E.S. Orwoll, S.L. Hui, T.M. Foroud, M. Peacock, M.J. Ecsons, Human ALOX12, but not ALOX15, is associated with BMD in white men and women, J. Bone Miner. Res. 21 (2006) 556–564.
[23] B. Fournier, S. Häring, A.M. Kaye, D. Sömjen, Stimulation of creatine kinase specific activity in human osteoblast and endometrial cells by estrogens and anti-estrogens and its modulation by calciotropic hormones, J. Endocrinol. 150 (1996) 275–285.
[24] D. Somjen, S. Katzburg, O. Sharon, A.M. Kaye, B. Gayer, F. Kohen, D. Hendel, G.H. Posner, Modulation of response to estrogens in cultured human female bone cells by a non-calcemic vitamin D analog: changes in nuclear and membranal binding, J. Steroid Biochem. Mol. Biol. 89–90 (2004) 393–395.
[25] F. Callewaert, M. Sinneseael, E. Gielen, S. Boonen, D. Vanderscueren, Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF1, and mechanical loading, J. Endocrinol. 207 (2010) 127–134.