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DT56a (Tofupill®/Femarelle™) selectively stimulates creatine kinase specific activity in skeletal tissues of rats but not in the uterus

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Abstract

The novel natural product DT56a (Tofupill®/Femarelle™), derived from soybean, has been shown to relieve menopausal vasomotor symptoms and to increase bone mineral density with no effect on sex steroid hormone levels or endometrial thickness. In the present study, we compared the effects of DT56a and estradiol-17β (E2) on bone and cartilage (Ep) of immature or ovariectomized female rats, by measuring the changes in the specific activity of the BB isozyme of creatine kinase (CK). Single short-term injection of high doses of DT56a induced estrogenic activity in bones and uterus similar to that of E2. When administered in multiple oral doses, DT56a stimulated skeletal tissues similarly to E2, but whereas E2 increased CK specific activity in the uterus, DT56a did not. The selective estrogen receptor modulator (SERM) raloxifene (Ral) blocked the stimulation of CK by either DT56a or by E2 in all tissues tested. Our findings suggest that DT56a acts as a selective estrogen receptor modulator stimulating skeletal tissues without affecting the uterus. The effect of DT56a on other systems, such as the vascular and the central nervous system, are currently under investigation.

Keywords: Phytoestrogens; Estradiol; Raloxifene; Bone; Cartilage; Uterus; DT56a; Creatine kinase

1. Introduction

Estrogen is well known for its beneficial effect on osteoporosis [1,2], a disease currently affecting more than 25 million women in the US alone, causing some 250,000 hip fractures each year. Osteoporosis is characterized by reduction in bone mineral density, with the result of fracture after minimal trauma. The effect of estrogen on a tissue is initiated by its binding to estrogen receptor (ER) in the responsive cell. The estrogen-ER complex is then translocated into the nucleus, where it binds to the DNA and modulates the rate of transcription of specific genes. Two ERs have been identified, ERα and ERβ, which differ in their structure and tissue distribution [3] and their biological effect [4]. The two key factors that control tissue selectivity of an estrogen are the structure of its receptor(s) and its interaction with co-regulators [5,6]. On the other hand, estrogen can also have non-desired effects that contribute to the development and growth of estrogen-dependent tumors, such as breast or uterine cancer [7]. These ominous side effects led to extensive research aimed at finding compounds with beneficial estrogenic effects on selected sites, such as bones (osteoporosis), [8] and cardiovascular system [9] without the harmful side effects. Two main groups of compounds are currently under intensive study: the pharmacological selective ER modulators (SERMs) [10,11] and phyto-estrogens [12,13].

SERMs function as estrogen agonists in some tissues and as antagonists in others. For example, the SERM tamoxifen acts as an antagonist of estrogen in breast tissue, and is therefore used to treat breast cancer, whereas in bone it acts as an estrogen agonist [14,15]. Raloxifene (Ral) functions as an estrogen agonist in bone and in the cardiovascular system and antagonist in breast cancer and in the uterus [16,17]. Phyto-estrogens as well, have been shown to have positive effects on skeletal tissues [18] and on vasomotor symptoms, with no effect on the uterus [19]. As an estrogenic marker we used the induction of creatine kinase (CK) specific activity [20]. The specific activity of the brain isozyme of CK is stimulated by estrogens both in vivo and in vitro, in tissues and cells containing active ER(s) [20–22], and therefore can be used as a marker of estrogen response.

Recent studies on phyto-estrogens as estrogenic compounds showed that the stimulatory effects on CK specific activity by estradiol-17β(E2) at 0.5 (μg per day per rat) and

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by licorice extract at 25 (μg per day per rat) were similar in the bone diaphysis and in the pituitary. Moreover, histomorphometric analysis of the diaphysis and epiphysis of the femoral bone showed that licorice and E2 had similar effects on trabecular bone volume and width, but not on cartilage width or the height of the growth plate [23]. Recent studies in our laboratory were carried out, using DT56a (Tofupill®/Femarelle™, Se–cure Pharmaceuticals, Yavne, Israel) a novel compound derived from soybean. Unlike the phyto-estrogens found in natural soybean and in most commercial compounds, those found in DT56a are primarily phyto-estrogenic aglycones. The chemical changes that take place during heating and the enzymatic processes that accompany the manufacture of Tofupill®, are similar to those that occur in the fermentation process of soy-based foods in the Asian diet [24]. DT56a has been shown to increase bone mineral density (Menopause, 2003, in press) and to relieve vasomotor symptoms of menopause with no effect on sex hormone levels or endometrial thickness [25].

In the present study, we compared the in vivo estrogenic properties of DT56a (Tofupill®) on rat skeletal tissues, using two types of rats. Immature rats and the previously described rat model for postmenopausal changes: ovariectomized female rats [26,27]. We found that DT56a displays estrogen agonistic activity in vivo but its repeated oral administration did not affect the uterus. This estrogenic activity was blocked by raloxifene.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade. Chemicals were purchased from Sigma (St. Louis, MO). DT56a (Tofupill®) was provided by Se-cure Pharmaceuticals, (Yavne, Israel), Raloxifene (Evista®) was extracted from commercially available tablets.

2.2. Animals

Immature female Wistar rats were used intact or were ovariectomized at the age of 25 days. Treatments were administered starting 2 weeks after surgery. The rats were housed in air-conditioned quarters with light from 05.00 to 19.00 h and were exposed to food and water ad libidum. All experiments were carried out according to the regulations of the Committee on Experimental Animals of the Tel-Aviv Sourasky Medical Center.

2.3. Creatine kinase extraction and assay

Changes in the specific activity of CK in epiphyseal cartilage (Ep), diaphyseal bone (Di) and uterus (Ut), induced by a single intraperitoneal injection (i.p.) of E2 or DT56a (1) (dissolved in 0.1% ethanol in saline, for both), were assayed in ovariectomized female rats, 2 weeks after surgery, or in 25-day-old immature female rats ($n = 5$ per group for each experiment). Matched control rats ($n = 5$) were injected with 0.05% ethanol in phosphate-buffered saline (PBS). E2 was injected at 5 μg for immature and 10 μg for ovariectomized female rats [17,21,22]. The rats were sacrificed 24 h after the injection. In multiple daily applications, rats were fed directly into the stomach daily for 4 days at the indicated doses.

The organs examined were removed and stored at −20 °C until processed for assay of creatine kinase activity as described previously [17,21,22]. Organs were homogenized in buffer as described previously [17,21,22]. Supernatant extracts were obtained by centrifugation of the homogenates at 14,000 × g for 5 min at 4 °C in an Eppendorf microcentrifuge. CK was determined by a coupled spectrophotometric assay described previously [17,21,22]. Protein was determined by Coomasie blue dye binding using bovine serum albumin as the standard [17,21,22].

2.4. Statistical analysis

Differences between the mean values obtained from the experimental and the control groups were evaluated by analysis of variance (ANOVA). A P-value <0.05 was considered significant.

3. Results

3.1. Stimulation of creatine kinase specific activity, by a single injection of DT56a or estradiol, into immature female rats

Immature female rats, weighing about 60 g, were injected with E2 (5 μg per rat) or increasing concentrations of DT56a (ranging from 64 μg per rat to 3200 μg per rat). These concentrations are equivalent, respectively, to 0.1–5 times the human experiments dosage, calculated on the basis of weight. DT56a and E2 were tested for their effects on the specific activity of CK in rat tissues (Ep, Di and Ut; Fig. 1). Stimulation of CK by DT56a in all organs was maximal at 1300 μg DT56a, with a significant increase relative to vehicle-treated controls evident by 130 μg DT56a. E2 stimulated CK similarly to the optimal dose of DT56a (Fig. 1).

3.2. Inhibition by raloxifene of the creatine kinase specific activity, stimulated by DT56a or by estradiol in immature female rats

Immature rats were injected with DT56a at the optimal dosage (1300 μg) or with E2 (5 μg), alone or together with 0.5 mg raloxifene (Ral). Ral, which is stimulatory in the skeletal tissues, completely blocked the stimulatory activity of both compounds in the Ep, but only to about half in diaphyseal bone (Di) (Fig. 2). In the uterus, the stimulatory effect of a single injection of DT56a was similar to that of

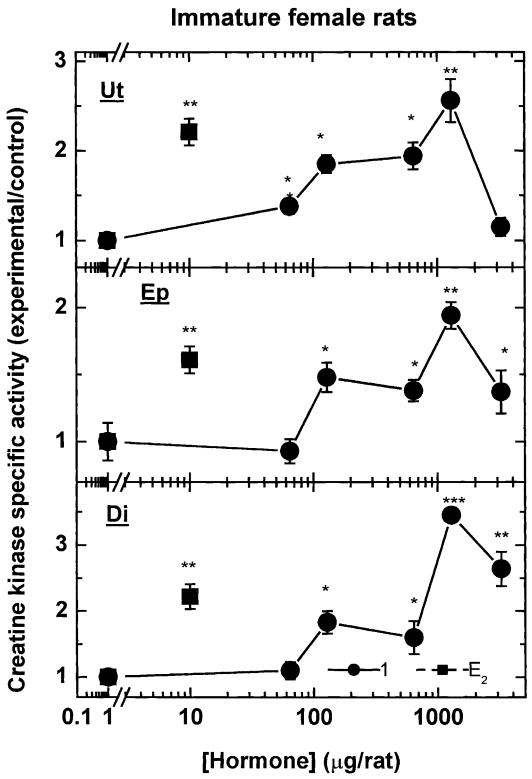

Fig. 1. Stimulation by a single injection of estradiol (E2) (5 μg) or of different concentrations of DT56a (1) on creatine kinase specific activity (CK) in epiphyseal cartilage (Ep), diaphyseal bone (Di) and in the uterus (Ut) of immature female rats. Details of treatment, extraction and analysis are given in Section 2. Results are means ± S.E.M. of $n = 5$ (10–15 assays from two to three experiments) and are expressed as the ratios between the specific activities of CK in hormone-treated and control animals. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The basal activity of creatine kinase was in Ep 1.13 + 0.16, in Di 1.30 + 0.03 and in Ut 0.34 + 0.02 μmol/(min mg) protein.

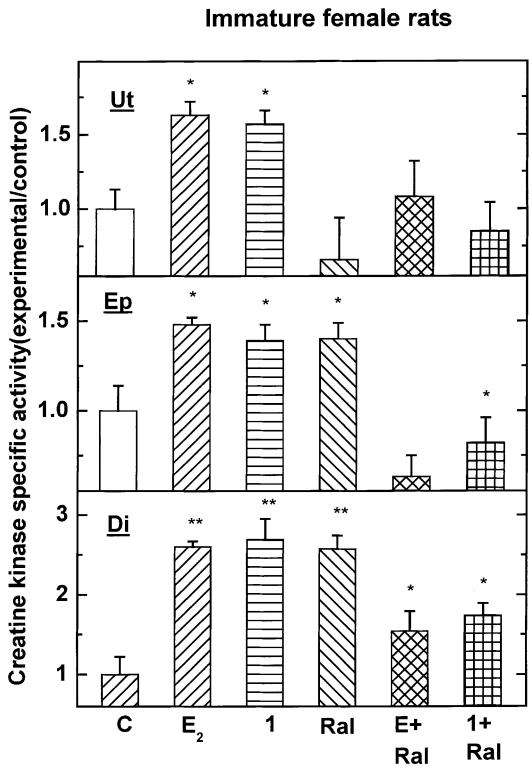

Fig. 2. Effect of a single injection of E2 (5 μg) or DT56a (1) (1300 μg, alone or in the presence of raloxifene (Ral) (0.5 mg), on creatine kinase specific activity (CK) in epiphyseal cartilage (Ep), diaphyseal bone (Di) and in the uterus (Ut) of immature female rats. Details for treatment, extraction and analysis are given in Section 2. Results are means ± S.E.M. of $n = 5$ (10 assays from 2–3 experiments) and are expressed as the ratios between the specific activities of CK in hormone-treated and control animals. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The basal activity of creatine kinase was in Ep 1.13 + 0.16, in Di 1.30 + 0.03 and in Ut 0.34 + 0.02 μmol/(min mg) protein.

E2. The effects of both compounds were blocked by Ral, which had no agonistic effect in the uterus (Fig. 2).

3.3. Stimulation of creatine kinase specific activity, by repeated oral administration of DT56a or estradiol in immature female rats

Daily feeding of DT56a (at a concentration of 650 μg, which is half of the optimal concentration injected), or E2 (5 μg) for 4 days stimulated CK specific activity in Ep and in Di (Fig. 3). In the Ut CK activity was stimulated to a significant extent by E2, whereas DT56a did not stimulate CK activity in the uterus (Fig. 3), different from the single injection shown earlier (Figs. 1 and 2).

3.4. Inhibition by raloxifene of the creatine kinase specific activity, stimulated by DT56a or estradiol injection in ovariectomized female rats

Ovariectomized female rats were injected with DT56a at the optimal concentration for adult rats (3200 μg), or with E2 (10 μg), alone or together with 0.5 mg of raloxifene. E2 and Ral stimulated enzyme activity similarly in both Ep and in Di (Fig. 4). DT56a was as active as E2 in Di, but half as active as E2 in Ep (Fig. 4). In the uterus, the stimulatory effect of a single injection of DT56a was slightly less active as was a single injection of E2 (Fig. 4). The effects of both compounds were blocked by Ral, which by itself did not stimulate CK in the uterus (Fig. 4).

3.5. Stimulation of creatine kinase specific activity, by repeated oral administration of DT56a or estradiol in ovariectomized female rats

Daily feeding of DT56a at a concentration of 1600 μg (which is half of the optimal concentration for adult rats), or of E2 (10 μg) for 4 days, stimulated CK specific activity in Ep and in Di (Fig. 5). The stimulation by DT56a in the skeletal tissues was about half of that of E2 (Fig. 5). No significant stimulation of DT56a was seen in Ut, which is significantly stimulated by E2 (Fig. 5).

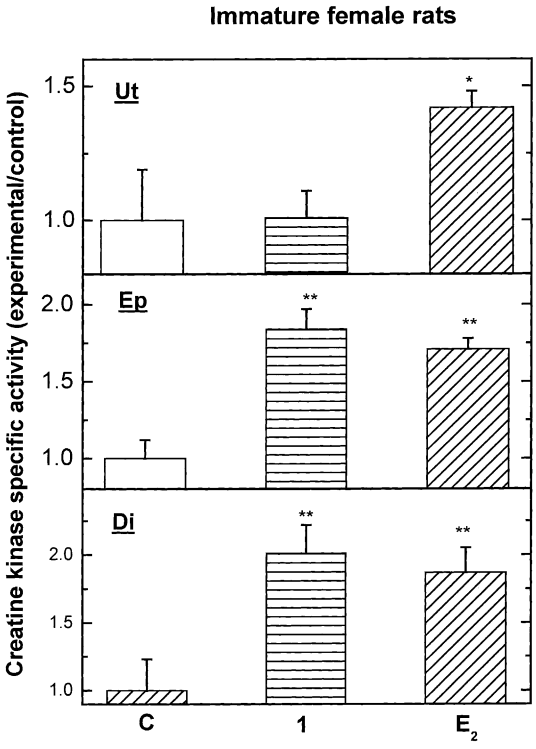

Fig. 3. Effects of multiple daily treatments (4 days) with E2 (5 μg) or with DT56a (1) (650 μg) on creatine kinase specific activity (CK) in epiphyseal cartilage (Ep), diaphyseal bone (Di) and in the uterus (Ut) of immature female rats. Details for treatment, extraction and analysis are given in Section 2. Results are means ± S.E.M. of $n = 5$ (10 assays from 2–3 experiments) and are expressed as the ratios between the specific activities of CK in hormone-treated and control animals. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The basal activity of creatine kinase was in Ep 1.13 + 0.16, in Di 1.30 + 0.03 and in Ut 0.34 + 0.02 μmol/(min mg) protein.

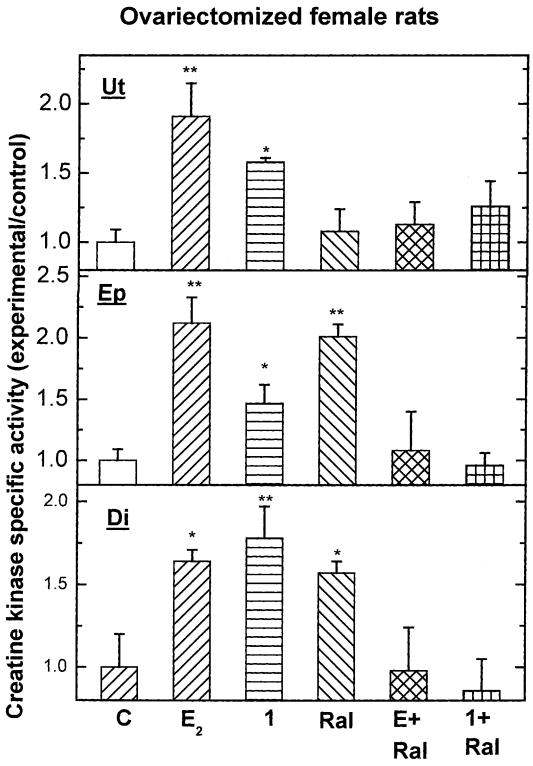

Fig. 4. Effects of a single injection of E2 (10 μg) or DT56a (1) (3200 μg), alone or in the presence of raloxifene (Ral; 0.5 mg), on creatine kinase specific activity (CK) in epiphyseal cartilage (Ep), diaphyseal bone (Di) and in the uterus (Ut) of ovariectomized female rats. Details for treatment, extraction and analysis are given in Section 2. Results are means ± S.E.M. of $n = 5$ (10 assays from 2–3 experiments) and are expressed as the ratios between the specific activities of CK in hormone-treated and control animals. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The basal activity of creatine kinase was in Ep 0.49 + 0.27, in Di 1.26 + 0.22 and in Ut 0.79 + 0.15 μmol/(min mg) protein.

4. Discussion

Phyto-estrogens are plant compounds with estrogen-like biological activity. They can be divided into three main subclasses: isoflavones, lignans, and coumestans [23,28,29]. The major lignans are enterolactone and enterodiol [30], the major coumestan is coumestrol, and the major isoflavonoids active in food are genistein and daidzein [31]. Epidemiological evidence indicates that intake of soybean (which is rich in isoflavonoids) is associated with lower breast cancer risk [32,33]. Genistein is reported to prevent cancellous bone loss and to maintain or increase bone density in postmenopausal women [34]. Examination of the binding of different phyto-estrogens to ER and of the effects of different phyto-estrogens, at a wide range of concentrations, on induction of PS2 (an estrogen-regulated antigen) and cell proliferation rate in human breast cancer cells showed that phyto-estrogens have very weak estrogenic activity, ranging from 500 to 15,000 times less than that of E2 [35]. Phyto-estrogens have been shown to differentiate between different ERs [3,24]. The data presented here, suggest that phyto-estrogens are not only able to mimic the function of E2 as physiological regulators of ER-expression, but also display tissue selectivity.

Soybean products, an important part of the diet in East Asian countries, contain significant amounts of the isoflavones daidzein and genistein, which may act as weak estrogens or as anti-estrogens. Genistein has been widely studied because of its broad spectrum of biological activities, including inhibition of tyrosine phosphorylation of proteins in the signal transduction pathway, inhibition of topoisomerase II, and induction of apoptosis and cell differentiation [36]. The factors determining whether the predominant activity of isoflavones is estrogenic or anti-estrogenic are quite complex. These compounds may be species- and/or tissue-specific and their effects may depend on concentration, duration of use and the method of administration [36].

We have used Tofupill®, a novel product derived from soybean. The active compound DT56a in Tofupill® is an enzymatic isolate of the active components found in the original soybean, with no alteration of the natural active composition, so that the beneficial quality of the original compound(s) is preserved. Tofupill® was shown to

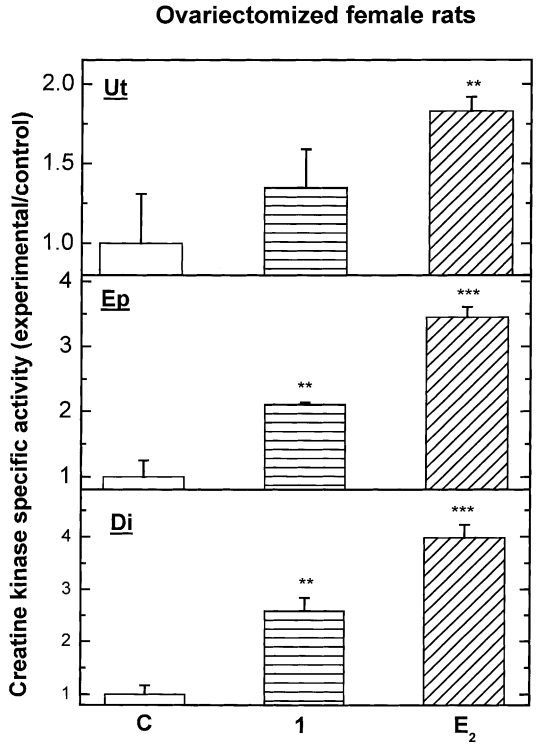

Fig. 5. Effect of multiple daily treatments (4 days) with E2 (10 μg) or with DT56a (1) (1600 μg) on creatine kinase specific activity (CK) in epiphyseal cartilage (Ep), diaphyseal bone (Di) and in the uterus (Ut) of ovariectomized female rats. Details for treatment, extraction and analysis are given in Section 2. Results are means ± S.E.M. of $n = 5$ (10 assays from 2–3 experiments) and are expressed as the ratios between the specific activities of CK in hormone-treated and control animals. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The basal activity of creatine kinase was in Ep 0.49 + 0.27, in Di 1.26 + 0.22 and in Ut 0.79 + 0.15 μmol/(min mg) protein.

relieve vasomotor symptoms of menopause with no effect on sex hormone levels or endometrial thickness [25]. Tofupill® significantly increased the bone mineral density of postmenopausal women relative to low-dose DT56a with calcium supplementation (Menopause 2003, in press).

In this study we examined the stimulation of CK specific activity by DT56a (Tofupill®) as a marker of estrogenic activity in skeletal tissues and correlated with bone formation [37,38]. When administered at high dosages by a single injection, CK specific activity induced by DT56a was in the same range as that of E2. However, whereas E2 stimulated CK activity in the uterus, DT56a at multiple doses did not. We found here that raloxifene, although active by itself, it antagonized E2, as well as DT56a activities. The phenomenon of mutual annihilation of action between 17β estradiol (E2) and a selective estrogen receptor modulator (SERM), such as raloxifene, previously described in prepubertal rat diaphysis, epiphysis, uterus and ROS 17/2.8 rat osteoblastic cells and in transiently co-transfected cells in culture. Therefore, SERMS can be full agonists when acting alone, but complete antagonists to a super-physiological dose of estrogen [39].

If this is due to desensitization or the inability to respond above certain fold, and if it depends on ERs or co-activators and/or co-repressors is still an open question. The inhibition by raloxifene of the stimulation of CK by both E2 and DT56a points to a common mechanism of action by these compounds, probably by utilizing the same ERs.

Since CK specific activity is only a marker for short-term effect of E2 on bone indicating later changes in bone formation [38], for direct measuring of skeletal tissue changes, we are currently testing the effect of DT56a on bone density and by histomorphology in long-term experiments. The effect of DT56a on the smooth–muscle vasculature is currently under investigation in an ongoing long-term study, since phyto-estrogens have been shown to have a positive effect on human vascular smooth muscle cells and in vasculature in vivo [38] the effect of DT56a on the smooth-muscle vasculature is currently under investigation in an ongoing long-term study. DT56a has the desirable characteristics of an effective and a safe selective natural estrogenic compound. It was shown to relieve menopausal symptoms [25], to elevate BMD with no changes in endometrial thickness or sex hormone profile (Menopause, 2003, in press). In the current study, it stimulated CK specific activity in skeletal tissues but not in the uterus. These findings enable DT56a to be considered a natural alternative for menopause.

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References

[1] R. Lindsay, J.C. Gallagher, M. Kleerekoper, J.H. Pickar, Effect of lower doses of conjugated equine estrogens with and without medroxyprogesterone acetate on bone in early postmenopausal women, J. Am. Med. Assoc. 287 (2002) 2668–2676.
[2] L. Speroff, J. Rowan, J. Symons, H. Genant, W. Wilborn, The comparative effect on bone density, endometrium, and lipids of continuous hormones as replacement therapy (CHART study). A randomized controlled trial, J. Am. Med. Assoc. 276 (1996) 1397–1403.
[3] G.G. Kuiper, J.G. Lemmen, B. Carlsson, J.C. Corton, S.H. Safe, P.T. van der Saag, B. van der Burg, J.A. Gustafsson, Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, Endocrinology 139 (1998) 4252–4263.
[4] E. Enmark, J.A. Gustafsson, Oestrogen receptors—an overview, J. Int. Med. 246 (1999) 133–138.
[5] F.C. Eng, A. Barsalou, N. Akutsu, N. Mercier, C. Zechel, S. Mader, J.H. White, Differential classes of coactivators recognize distinct but overlapping binding sites on the estrogen receptor ligand binding domain, J. Biol. Chem. 273 (1998) 28371–28377.
[6] C.L. Smith, Z. Nawaz, B.W. O'Malley, Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen 4-hydroxy tamoxifen, Mol. Endocrinol. 11 (1997) 657–666.
[7] D.M. Philips, L. Balducci, Current management of breast cancer, Am. Fam. Physician 53 (1996) 657–665.

[8] D.B. Muchmore, Raloxifene: a selective estrogen receptor modulator (SERM) with multiple target system effects, Oncologist 5 (2000) 388–392.
[9] E. Barrett-Connor, D. Grady, A. Sashegyi, P.W. Anderson, Raloxifene and cardiovascular events in osteoporotic postmenopausal women: 4-year results from the MORE (multiple outcomes of raloxifene evaluation) randomized trial, J. Am. Med. Assoc. 287 (2002) 847–857.
[10] C.E. Kearney, D.W. Purdie, Selective estrogen receptor modulators, Climacteric 1 (1998) 143–147.
[11] C. Gajdos, V.C. Jordan, Selective estrogen receptor modulators as a new therapeutic drug group: concept to reality in a decade, Clin. Breast Cancer 2 (2002) 272–281.
[12] D.M. Tham, C.D. Gardner, W.L. Haskell, Potential health benefits of dietary phyto-estrogens: a review of the clinical, epidemiological, and mechanistic evidence, J. Clin. Endocrinol. Metab. 83 (1998) 2223–2235.
[13] K.D.R. Setchell, Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones, Am. J. Clin. Nutr. 68 (Suppl) (1998) 1333S–1346S.
[14] B. Breuer, S. Wallenstein, R. Anderson, Effects of tamoxifen on bone fractures in older nursing home residents, J. Am. Geriatr. Soc. 46 (1998) 968–972.
[15] D.P. McDonnell, C.E. Connor, A. Wijayaratne, C.Y. Chang, J.D. Norris, Definition of the molecular and cellular mechanisms underlying the tissue-selective agonist/antagonist activities of selective estrogen receptor modulators, Recent Prog. Horm. Res. 57 (2002) 295–316.
[16] S.L. Silfen, A.V. Ciaccia, H.U. Bryant, Selective estrogen receptor modulators: tissue selectivity and differential uterine effects, Climateric 2 (1999) 268–283.
[17] D. Somjen, A. Waisman, A.M. Kaye, Tissue selective action of tamoxifen methiodide, raloxifene and tamoxifen on creatine kinase B activity in vitro and in vivo, J. Steroid Biochem. Mol. Biol. 59 (1996) 389–396.
[18] S.M. Potter, J.A. Baum, H. Teng, R.J. Stillman, N.F. Shay, J.W. Erdman Jr., Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women, Am. J. Clin. Nutr. 68 (Suppl) (1998) 1375S–1379S.
[19] A.A. Ewies, Phytoestrogens in the management of the menopause: up-to-date, Obstet. Gynecol. Surv. 57 (2002) 306–313.
[20] S.D. Malnick, A. Shaer, H. Soreq, A.M. Kaye, Estrogen-induced creatine kinase in the reproductive system of the immature female rat, Endocrinology 113 (1983) 1907–1909.
[21] 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) 3361–3365.
[22] D. Somjen, A. Waissman, Y. Weisman, A.M. Kaye, Nonhypercalcemic analogs of vitamin D stimulate creatine kinase B activity in osteoblast-like ROS 17/2.8 cells and up-regulate their responsiveness to estrogens, Steroids 63 (1998) 340–343.
[23] S. Tamir, M. Eizenberg, D. Somjen, S. Izrael, J. Vaya, Estrogen-like activity of glabrene and other constituents isolated from licorice root, J. Steroid Biochem. Mol. Biol. 78 (2001) 291–296.
[24] H.J. Wang, P.A. Murphy, Mass balance study of isoflavones during soybean processing, J. Agric. Food Chem. 44 (1996) 2377–2383.
[25] I. Yoles, B. Kaplan, D.S. Seidman, B. Chayen, Phytoestrogens: a time-tested solution for menopausal symptoms revisited, Obstet. Gynecol. 99 (Suppl) (2002) 58S.
[26] R.T. Turner, J.J. van der Steenhover, N.H. Bell, The effect of ovariectomy and 17b-estradiol on bone histomorphometry in growing rats, J. Bone Mineral. Res. 2 (1987) 115–122.
[27] D. Somjen, Z. Mor, A.M. Kaye, Age dependence and modulation by gonadectomy of the sex-specific response of rat diaphyseal bone to gonadal steroids, Endocrinology 134 (1994) 809–814.
[28] S. Tamir, M. Eizenberg, D. Somjen, S. Izrael, J. Vaya, Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cells, Cancer Res. 60 (2000) 5704–5709.
[29] J. Vaya, S. Tamir, D. Somjen, Estrogen-like activity of licorice root extract and its constituent (in preparation).
[30] J. Liggins, R. Grimwood, S.A. Bingham, Extraction and quantification of lignan phytoestrogens in food and human samples, Anal. Biochem. 287 (2000) 102–109.
[31] H. Wiseman, The therapeutic potential of phytoestrogens, Exp. Opin. Investig. Drugs 9 (2000) 1829–1840.
[32] H.P. Lee, l. Gouerly, S.W. Duffy, J. Esteve, J. Lee, N.E. Day, Dietary effects on breast-cancer risk in Singapore, Lancet 337 (1991) 1197–1200.
[33] B.F. Fournier, J.W. Erdman Jr., G.B. Gordon, Soy, its components and cancer prevention: a review of the in vitro, animal and human data, Cancer Epidemiol. Biomarkers Prev. 7 (1998) 1055–1065.
[34] M. Valente, L. Buflino, G.N. Castiglione, R. D'Angelo, A. Mancuso, P. Galoppi, X. Zichella, Effects of 1-year treatment with isoipriflavone on bone in post-menopausal women with low bone mass, Calcif. Tissue Int. 54 (1994) 377–380.
[35] L. Markiewicz, J. Garey, H. Adlerkreutz, E. Gurpide, In vitro bioassays of nonsteroidal phytoestrogens, J. Steroid Biochem. Mol. Biol. 45 (1993) 399–405.
[36] S. Barnes, B. Boersma, R. Patel, M. Kirk, V.M. Darley-Usmar, H. Kim, J. Xu, Isoflavonoids and chronic disease: mechanisms of action, Biofactors 12 (2000) 209–215.
[37] A.M. Kaye, T.-Y. Kim, F. Kohen, D. Somjen, Anabolic effects of estrogen and parathyroid hormone on skeletal tissues: the use of creatine kinase B activity as a response marker, Arch. Gerontol. Geriatr. 24 (1997) 197–209.
[38] T.-Y. Kim, V. Vargas, H. Mayer, D. Somjen, A.M. Kaye, Selective anabolic effects of muteins of mid-region PTH fragments on skeletal tissues of prepubertal rats, Bone 30 (2002) 78–84.
[39] A.M. Kaye, M. Spatz, A. Waisman, S. Sasson, S. Tamir, J. Vaya, D. Somjen, Paradoxical interactions among estrogen receptors, J. Steroid Biochem. Mol. Biol. 76 (2001) 85–93.