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Diabetes Modulates Differentially Creatine Kinase-Specific Activity Responsiveness to Estradiol-17β and to Raloxifene in Rat Organs

Dalia Somjen,[1]* Michal Shen,[2] Naftali Stern,[1] and Nitsa Mirsky[2]

[1]Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center and The Sackler Faculty of Medicine, Tel-Aviv University; Tel-Aviv 64239, Israel
[2]Faculty of Science, Haifa University Har-Hacarmel, Haifa 31905, Israel

Abstract Diabetes mellitus increases the risk for CVD in women. While there is considerable evidence suggesting beneficial effects of estrogen on decreasing lipid peroxidation, atherosclerotic processes, and cardiovascular diseases, diabetes negates most estrogen protective effects as well as the skeletal protective effects of estrogens, which are not discernable in diabetic women. In the present study, we examined the in vivo effects of estradiol-17β (E_2), on creatine kinase (CK)-specific activity, in estrogen-responsive organs from healthy and diabetic rats. Healthy or diabetic (streptozotocin-induced) female rats were injected with either E_2 (10–50 μg/rat) or raloxifene (Ral; 500–1,000 μg/rat). Twenty-four hours following the injection, animals were sacrificed; their organs removed and assayed for CK-specific activity. CK-specific activity in different organs [Left ventricle of heart (Lv), uterus (Ut), aorta (Ao), para uterine adipose tissue (Ad), epiphyseal cartilage (Ep), and diaphyseal bone (Di)] from healthy animals, was stimulated with increased doses of E_2, with maximum at 20 μg/rat. Age-matched diabetic female rats exhibited a remarkable decreased response to E_2 in all organs except Ut. In contrast, the response to Ral was not altered in diabetic rats. Similar results were observed in organs from ovariectomized female rats (Ovx), healthy or diabetic. These results support our previous in vitro findings, demonstrating that hyperglycemia decreases CK response to E_2 but not to Ral in cultured human vascular and bone cells. In summary, diabetes mellitus decreases CK response to E_2 but not that of Ral in skeletal and vascular tissues. The decreased response to E_2 detected in organs derived from diabetic rats might be due to changes in nuclear and/or membrane estrogen receptors and/or other genomic and non-genomic pathways, as was shown in in vitro cellular models. J. Cell. Biochem. 99: 133–139, 2006.

Key words: estradiol-17β; raloxifene; diabetes; bone; vascular organs; uterus; pituitary; creatine kinase; hyperglycemia

Cardiovascular diseases (CVD) are the leading cause of mortality in post-menopausal women, which is increased by loss of female sex hormones after menopause [Nabulsi et al., 1993]. Although CVD is less common in pre-menopausal women, diabetes mellitus greatly increases the risk for it in these women. Moreover, estrogen replacement therapy (ERT), which reduces the incidence of CVD in post-menopausal healthy women [Nabulsi et al., 1993; Koh et al., 1997], is ineffective in post-menopausal diabetic women [Koh et al., 1997; Node et al., 1997]. Gender-related protection from atherosclerosis in pre-menopausal women is apparently attributable to estradiol's multiple favorable interactions with the arterial wall, lipid metabolism, and fibrinolytic system [Kannel and McGee, 1979; Williams et al., 1997]. Several studies have indicated that a pre-menopausal status does not confer cardiovascular [Kaseta et al., 1999] or bone protection [He et al., 2004] in diabetic women. Moreover, recent evidence suggests that hormone replacement therapy induces less endothelial-dependent vasodilatation in the microcirculation in diabetic women compared with healthy post-menopausal female patients [Yamauchi et al., 1990; Kaseta et al., 1999].

Estrogen deficiency also causes post-menopausal osteoporosis [Cosman and Lindsay, 1999; Manolagas et al., 2002], which affects every third woman above the age of 65.

*Correspondence to: Dalia Somjen, PhD, Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center, 6 Weizman St. Tel-Aviv, 64239, Israel. E-mail: dalias@tasmc.health.gov.il

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Osteoporosis is characterized by a reduction in bone density and bone strength, to the extent that fractures occur after minimal trauma. At menopause, an accelerated loss of bone mass (3%/year) takes place during the first 5 years, along with changes in bone structure. It has been suggested that, estrogen stimulates osteoblastic proliferation and activity, decreases the number of osteoclasts, slows the rate of bone remodeling, and thus protects against bone loss. The skeletal protective effects of E_2 are not discernable in diabetic women in whom osteoporosis evolves and progresses during the reproductive years at a rate comparable to that seen in post-menopausal women [He et al., 2004].

The effect of estrogen on a tissue is initiated by its binding to estrogen receptors (ERs) in the responsive cell. Two ERs have been identified, ERα and ERβ, which differ in their structure and tissue distribution [Enmark and Gustafsson, 1999] and their biological effect [Manolagas et al., 2002]. The two key factors that control tissue selectivity for estrogen are the nature of its receptor(s) and its interaction with coregulators [Shao et al., 2004; Talukder et al., 2004].

We have previously studied the effects of estrogens in a rat model [Somjen et al., 2000, 2001b], using the increase in the specific activity of creatine kinase (CK) as a response marker. The brain type (BB) isozyme of CK, part of the "energy buffer" system which regulates the cellular concentration of ATP and ADP, is the major component of the "E_2-induced protein" of rat uterus [Reiss and Kaye, 1981] and other tissues containing ERs [Malnick et al., 1983].

We have reported that E_2 exerts a stimulatory effect on CK-specific activity in rat aorta, representing both endothelial and smooth muscle cells, and left ventricle of the heart representing smooth muscle cells, via specific receptors, parallel to the effects in human vascular cells in culture [Somjen et al., 1998, 2001a]. This effect of E_2 may favorably affect vascular response to injury, in that it is consistent with better capacity for re-endothelialization along with attenuation of post-injury myointimal proliferation. CK stimulation is an efficient response marker to detect the activity of E_2 also in vascular cells [Somjen et al., 1998, 2001a] and in bone cells [Fournier et al., 1996] which contain low concentrations of ERs [Eriksen et al., 1988; Komm et al., 1988]; notably, the stimulation of CK in cultured bone cells, correlates with increased DNA synthesis in bone, requires the higher end of the physiological range of estrogen concentrations [Somjen et al., 1989]. We have established a primary human bone-derived osteoblast-like cell culture system, exhibiting major osteoblastic characteristics, and responded to gonadal steroids sex specifically [Katzburg et al., 1999, 2001]. We used the stimulation of CK as a response marker for osteoblast-like cells containing ERs [Katzburg et al., 1999; Somjen et al., 2000].

We also found that the presumably favorable effects of E_2 on CK activity, for both osteoblasts [Somjen et al., 2005a] and vascular cells [Somjen et al., 2004] are not operative under hyperglycemia. Human bone and vascular cells have both specific nuclear and membranal binding sites for estrogenic compounds, which are modulated by hyperglycemia leading to altered hormonal responsiveness. Thus, high glucose blocks important effects of E_2, which are likely contributors to bone preserving properties and the beneficial effects on the vascular system.

Raloxifene (Ral), which exhibits estrogen-mimetic effects on bone and vascular cells [Fournier et al., 1996; Somjen et al., 2001a], was not affected by hyperglycemia, as we previously found for human vascular and bone cells [Somjen et al., 2004, 2005a]. Moreover, there is evidence on Ral favorable effects on lipoproteins in diabetic women. Thus, the use of Ral may circumvent the loss of estrogen responsiveness induced by hyperglycemia in diabetic women.

In the present study we examined the possibility that the favorable effects of E_2 on bone and vascular tissues compared to other responsive tissues which contain ERs, are not operative in diabetic immature or ovariectomized female rats. We tested the hypothesis that chronic hyperglycemia attenuates the stimulatory effects of E_2 in comparison to Ral on CK activity in female-derived skeletal and vascular tissues as well as other responsive tissues.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade. Chemicals, 17β-estradiol (E_2), and the CK assay

kit were purchased from Sigma Chemicals Co. (St. Louis, MO). Ral was the gift of Dr. B. Fournier (Ciba-Geigy, Basel, Switzerland).

Animals and Induction of Diabetes

Sprague Dawley female rats at the age of 5 weeks, weighing 120 g, were injected subcutaneously with a single dose of Streptozotocin (STZ; 60 mg/Kg BW in 0.05 M citrate buffer, pH 5.7). Additional group of animals were injected with the vehicle (0.05 M citrate buffer, pH 5.7), and served as healthy controls. The animals were kept for 8 weeks in cages with 12 h cycles of light and dark, Purina chow and tap water supplemented ad libidum. Rats were used either as intact or 2 weeks post ovariectomy (OVX).

Hormonal Treatment

E_2 was injected at 10–50 μg/immature female rats and 20 μg/OVX female rats, and 500 μg or 1,000 μg Ral for the above groups of animals respectively, for 24 h, followed by harvesting the different organs indicated for CK assay.

Creatine Kinase Activity Assay

Organs were homogenized in cold isotonic extraction buffer [Somjen et al., 2000]. Supernatant extracts were obtained by centrifugation at 14,000*g* for 5 min at 4°C in an Eppendorf micro centrifuge. CK activity was assayed in a Kontron Model 922 Uvicon Spectrophotometer at 340 nm using a Sigma coupled assay kit (procedure 47-UV).

Statistical Significance

The significance of differences between experimental and control values was evaluated using a non-paired, two-tailed Student's *t*-test in which n = number of donors.

RESULTS

Modulation of CK-Specific Activity in Intact and OVX Healthy and Diabetic Female rat Organs

STZ treatment of intact female rats modulates constitutive CK-specific activity in different organs (Fig. 1). Aorta (Ao), uterus (Ut), and pituitary (Pi), are not affected ($-13 \pm 13\%$, $-9 \pm 20\%$, and $-27 \pm 14\%$, respectively) while it is increased in left ventricle (Lv), epiphysis (Ep), diaphysis (Di), and adipose tissue (Ad): $42 \pm 19\%$, $46 \pm 21\%$, $42 \pm 16\%$, and $60 \pm 25\%$ $P < 0.05$, respectively. In OVX, similar results were obtained: Ao, Ut, and Pi are not affected

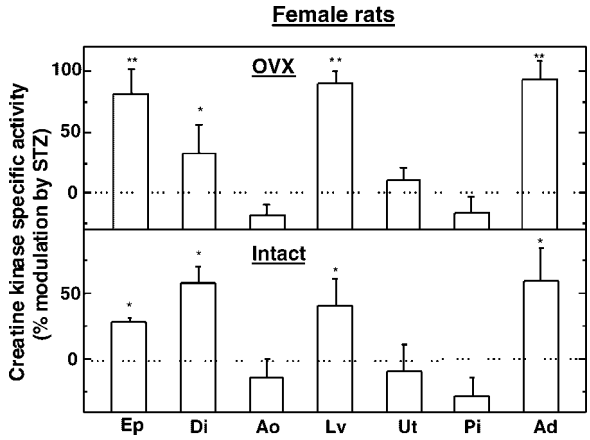

Fig. 1. Constitutive levels of CK-specific activity in different organs from STZ-treated non-OVX (intact) (**lower panel**) and Ovx (**upper panel**) female rats. Rats were treated and assayed for CK activity as described in Materials and Methods. Results are mean ± SEM for n = 5–15 rats/group. Experimental means compared to control means: $^{*}P < 0.05$ and $^{**}P < 0.01$. Basal activity in organs from intact rats are: in Ep, epiphysis: 0.73 + 0.06 μmol/min/mg protein; Di, diaphysis: 0.84 + 0.13 μmol/min/mg protein; Ao, aorta: 0.22 + 0.03 μmol/min/mg protein; Lv, left ventricle: 1.06 + 0.16 μmol/min/mg protein; Ut, uterus: 0.72 + 0.09; Pi, pituitary: 1.12 + 0.17 μmol/min/mg protein; Ad, para uterine adipose tissue: 0.32 + 0.07 μmol/min/mg protein. Enzyme activity levels in organs from Ovx rats are: in Ep: 0.71 + 0.15, Di: 0.33 + 0.05, Ao: 0.29 + 0.04, Lv: 0.78 + 0.18, Ut: 0.51 + 0.05, Pi: 0.47 + 0.02, and Ad: 0.22 + 0.05 μmol/min/mg protein.

($20 \pm 10\%$, $10 \pm 8\%$, and $18 \pm 15\%$, respectively) whereas an increase in Lv, Ep, Di, and Ad ($90 \pm 5\%$, $80 \pm 20\%$, $35 \pm 20\%$, and $90 \pm 20\%$, respectively) was found.

Dose-Dependent Stimulation of CK-Specific Activity in Intact and Diabetic Non-OVX (intact) Female Rat Organs by Estradiol-17β

Non-OVX (intact) female rats either intact or STZ-injected, treated with E_2 10–50 μg/rat for 24 h, showed a significant increase in CK activity in organs from intact rats (Fig. 2) to different extent. In all organs, except the uterus, diabetes abolished or significantly decreased CK-specific activity in response to E_2 (Fig. 2).

The Effect of Estradiol −/+ B or Raloxifene on CK-Specific Activity in Non-OVX (intact) Healthy and Diabetic Female Rat Organs

Non-OVX (intact) female rats either intact or STZ injected, treated with 500 μg/rat of Ral for

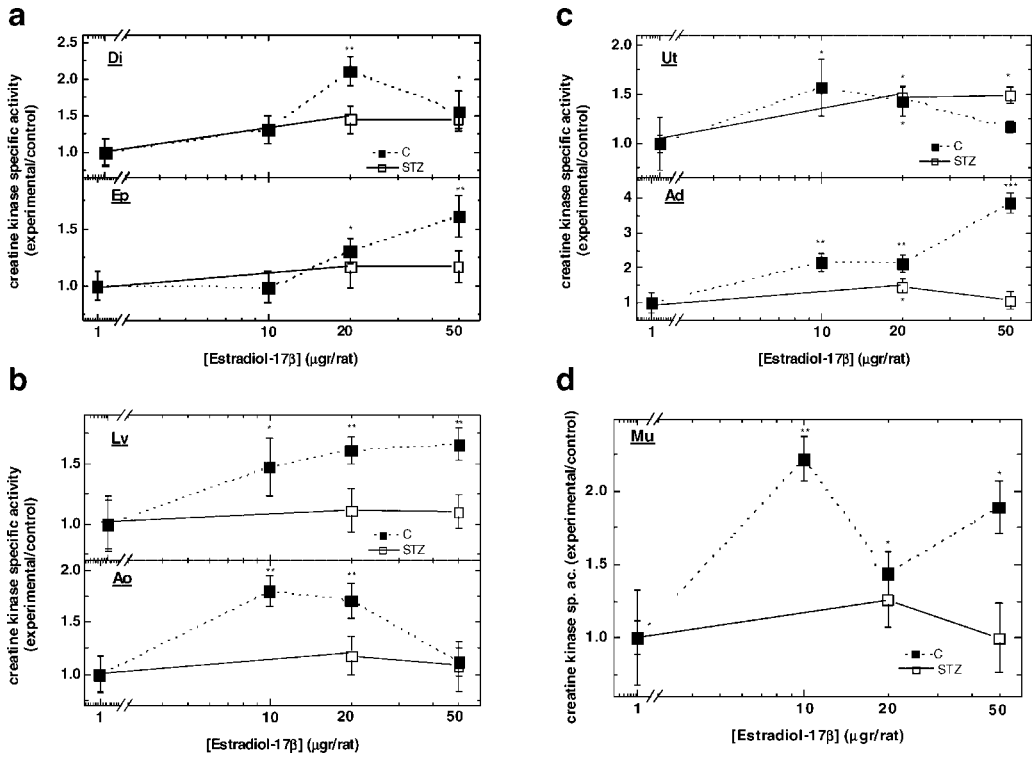

Fig. 2. Dose-dependent stimulation of CK-specific activity by E_2 in Ep and Di (**a**), Ao and Lv (**b**), Ut and Ad (**c**) and in skeletal muscle (Mu) (**d**) from non-OVX (intact) female rats, either intact or STZ-injected animals. Rats were treated and assayed for CK activity as described in Materials and Methods. Results are mean ± SEM for n = 5–10 rats/group for the different treatments. Experimental means compared to control means: $^{*}P < 0.05$; $^{**}P < 0.01$.

24 h, showed a significant increase in CK activity in organs from intact rats except Ut and Ad (Figs. 3 and 5) to different extent. In all responsive organs, except Ut and Ad, the response to Ral was not abolished by diabetes (Figs. 3 and 5).

The Effect of Estradiol-17β or Raloxifene on CK-Specific Activity in Ovariectomized Healthy and Diabetic Female Rat Organs

Ovariectomized female rats (Ovx) either intact or STZ-injected, treated with E_2 for 24 h, showed a significant increase in CK activity in different organs from intact rats (Figs. 4 and 5) to different extent. In all organs, diabetes produced abolishment or significant decrease of CK-specific activity in response to E_2 (Figs. 4 and 5). The decrease in the response of the uterus to E_2 is significantly much smaller compared with other organs. However, diabetes did not abolish the response to Ral in all responsive organs, except Ut and Ad (Figs. 4

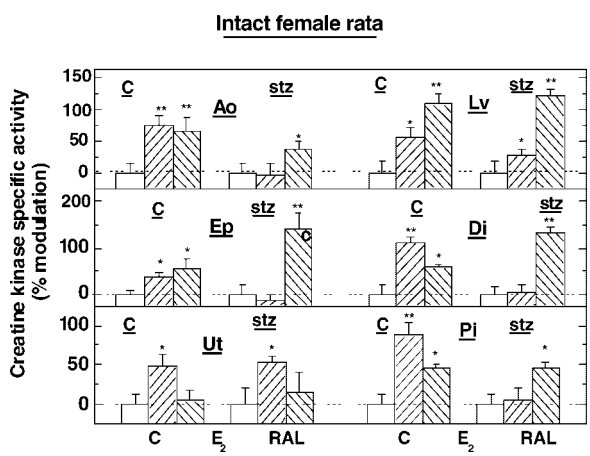

Fig. 3. Stimulation of CK activity by E_2 or RAL in Ao and Lv (**upper panel**), Ep and Di (**middle panel**), and Ut and Pi (**lower panel**) from non-OVX (intact) female rats, either intact or STZ-injected animals. Rats were treated with E_2 or Ral and assayed for CK activity as described in Materials and Methods. Results are mean ± SEM for n = 5–10 rats/group for the different treatments. Experimental means compared to control means: $^{*}P < 0.05$; $^{**}P < 0.01$.

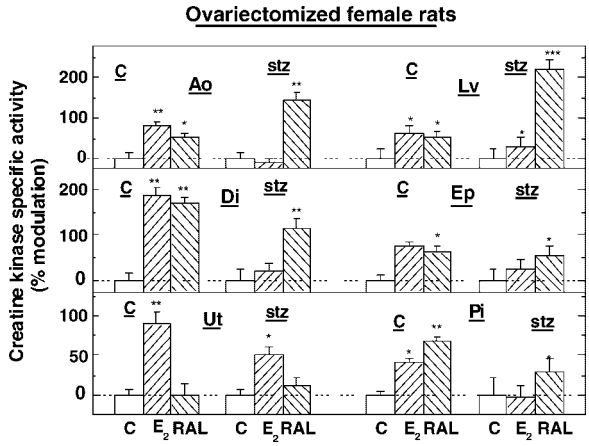

Fig. 4. Stimulation of CK activity by E_2 or Ral in Ao and Lv (**upper panel**) Ep and Di (**middle panel**), and Ut and Pi (**lower panel**) from OVX female rats, either intact or STZ-injected animals. Rats were treated and assayed for CK activity as described in Materials and Methods. Results are mean ± SEM for n = 5–10 rats/group for the different treatments. Experimental means compared to control means: $^*P < 0.05$; $^{**}P < 0.01$.

and 5), which did not respond to Ral in both intact and diabetic stage.

DISCUSSION

We demonstrated in the present study that chronic exposure of rat organs to high glucose concentrations induced by diabetes, augmented the levels of CK-specific activity in Ep, Di, Lv, and Ad but not in Ut, Ao, and Pi from both intact and Ovx animals. This is in accord with earlier

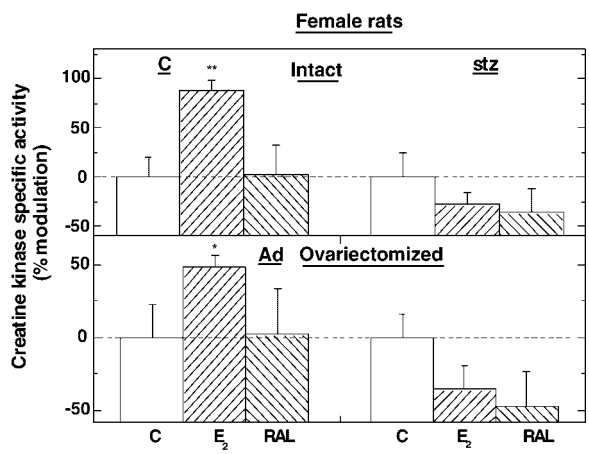

Fig. 5. Stimulation of CK activity by E_2 or Ral in Ad from non-OVX (intact) (**upper panel**) and Ovx (**lower panel**) female rats, either intact or STZ-injected animals. Rats were treated and assayed for CK activity as described in Materials and Methods. Results are mean ± SEM for n = 5–10 rats/group for the different treatments. Experimental means compared to control means: $^*P < 0.05$; $^{**}P < 0.01$.

reports, that short-term incubation in a high glucose medium increases porcine, rabbit, and human vascular smooth muscle cells (VSMC) replication [Natarajan et al., 1992; Orchard et al., 1999; Aveno et al., 2000]. Moreover, diabetic status substantially modified the effects of estradiol-17β on all rat organs tested except Ut, which was affected to a lower extent. In contrast, high glucose did not block the effects of Ral in the different organs studied.

Previously, we showed that hyperglycemia modulates the effects of estrogen but not Ral on DNA synthesis and on CK activity in human VSMC and ECV 304 cells [Somjen et al., 2004]. These effects on human vascular cells' response to estrogens are probably modulated via non-classical membranal estrogen binding sites and not by nuclear receptors [Somjen et al., 2004, 2005a].

In order to understand the mechanism of the changes induced by hyperglycemia, we showed that the abolition of estrogenic stimulation by hyperglycemia occurs also in our non-transformed human-derived primary osteoblasts, was accompanied in contrast, by increases in mRNA levels of ERα and to a lesser extent in ERβ in female cells at different ages [Somjen et al., 2005a]. We also demonstrated total cellular (mainly nuclear) and membranal estrogen binding to the different cells. While in normal human osteoblasts, estrogens were bound to both nuclear and membranal sites; Ral was bound only to nuclear binding sites. This parallels other findings [Aveno et al., 2000; Somjen et al., 2004, 2005b] using human vascular smooth muscle cells, where Ral showed no binding to membranal sites and did not prevent estrogen-mediated inhibition of cell proliferation. Attempt to correlate ERs mRNAs with the changes in nuclear and/or membrane binding failed also in these vascular cells [Somjen et al., 2004, 2004a, 2005b].

We also demonstrated changes in ERs mRNA expression by real time PCR, while ERα and ERβ mRNA were found in female-derived bone cells, hyperglycemia increased ERα and ERβ expression in female-derived cells [Somjen et al., 2005a]. The modulation of ERs is a recent addition to the spectrum of changes induced by hyperglycemia [Somjen et al., 2004, 2004a, 2005a], which stimulates the differentiation of osteoblasts and osteoclasts and stimulates osteoblasts to produce osteocalcin and alkaline phosphatase.

Bone growth is disturbed in diabetes [Goodman and Hori, 1984; He et al., 2004] and is also not enhanced to the same extent compared with healthy women, by hormone replacement therapy [Orchard et al., 1999]. This might be the result of lower hip BMD in young women due to their type one diabetes [Liu et al., 2003].

ERT, which reduces the incidence of CVD in post-menopausal healthy women [Nabulsi et al., 1993; Koh et al., 1997], is also ineffective in post-menopausal diabetic women [Koh et al., 1997; Node et al., 1997]. The use of Ral might provide an alternative solution for HRT in diabetic women. Further studies in this direction in animal models have to be conducted.

REFERENCES

Aveno R, Arora S, Carnody BJ, Cosby K, Sidawy AN. 2000. Thiamine (Vitamin B_1) Protects against glucose-and insulin-mediated proliferation of human infragenicular arterial smooth muscle cell proliferation. Amer Vasc Surg 14:37–43.

Cosman F, Lindsay R. 1999. Selective estrogen receptor modulators: Clinical spectrum. Endocr Rev 20:418–434.

Enmark E, Gustafsson JA. 1999. Oestrogen receptors—An overview. J Inter Med 246:133–138.

Eriksen EF, Colard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC, Riggs BL. 1988. Evidence of estrogen receptors in normal human osteoblast-like cells. Science 241:84–86.

Fournier B, Haring S, Kaye AM, Somjen D. 1996. Stimulation of creatine kinase specific activity in human osteoblast and endometrial cells by estrogens and antiestrogens and its modulation by calciotropic hormones. J Endocrinol 150:275–285.

Goodman W, Hori M. 1984. Diminished bone formation in experimental diabetes. Relationship to osteoid maturation and mineralization. Diabetes 33:825–831.

He H, Liu R, Desta T, Leon C, Gerstenfeld LC, Graves DT. 2004. Diabetes causes decreased osteoclastogenesis, reduced bone formation and enhanced apoptosis of osteoblastic cells in bacteria stimulated bone loss. Endocrinology 145:447–452.

Kannel WB, McGee DL. 1979. Diabetes and cardiovascular disease: The Framingham Study. J Amer Med Assoc 241:2038–2038.

Kaseta JR, Skofar DF, Ram JL, Sowers JR. 1999. Cardiovascular disease in the diabetic women. J Clin Endocrinol Metab 84:1835–1838.

Katzburg S, Lieberherr M, Ornoy A, Klein BY, Hendel D, Somjen D. 1999. Isolation and hormonal responsiveness of primary cultures of human bone-derived cells: Gender and age differences. Bone 25:667–673.

Katzburg S, Ornoy A, Hendel D, Lieberherr M, Kaye AM, Somjen D. 2001. Age and gender specific stimulation of creatine kinase specific activity by gonadal steroids in human bone-derived cells in culture. J Endocrinol Invest 24:166–172.

Koh KK, Mincemoyer R, Bui MN, Csako G, Pucino F, Gkuetta V, Waclwin M, Cannon RO. 1997. Effects of hormone replacement therapy on fibrinolysis in postmenopausal women. New England J Med 336:683–690.

Komm BS, Terpening CM, Benz DJ, Graeme KA, Kox M, Greene GL, Gallegos A, O'Malley BW, Houssler MR. 1988. Estrogen binding, receptor mRNA, and biological response in osteoblast-like osteosarcoma cells. Science 241:81–84.

Liu EY, Wactawaski-Wende J, Donhaue RP, Dmochowski J, Hovey KM, Quattrin T. 2003. Does low bone mineral density start in post-teenage years in women with type 1 diabetes? Diabetes Care 26:2365–2369.

Malnick SDH, Shaer A, Soreq H, Kaye AM. 1983. Estrogen induced creatine kinase in the reproductive system of the immature female rat. Endocrinology 19:1907–1909.

Manolagas SC, Kousteni S, Jilka RL. 2002. Sex steroids and bone. Rec Progr Hormone Res 57:385–409.

Nabulsi AA, Folsom AR, White A, Patsch W, Heiss G, Wu KK, Szklo M. 1993. Association of hormone replacement therapy with various cardiovascular risk factors in postmenopausal women: The Atherosclerosis Risk in Communities Study investigations. New England J Med 28:1069–1075.

Natarajan R, Gonzales N, Xu L, Nadler J. 1992. Vascular smooth muscle cells exhibit increased growth response to elevated glucose. Biochem Biophys Res Commun 87: 552–560.

Node K, Kitakaze M, Kosaka H, Minamino T, Funaya H, Hori H. 1997. Amelioration of ischemia and reperfusion-induced myocardial injury by 17β-estradiol: Role of nitric oxide and calcium-activated potassium channels. Circulation 96:1953–1963.

Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, LaPorte RE, Kuller LH. 1999. Prevalence of complications in IDDM by sex and duration: Pittsburgh Epidemiology of Diabetes Complications Study II. Diabetes 39:1116–1124.

Reiss N, Kaye AM. 1981. Identification of the major component of the estrogen induced protein in rat as the BB isozyme of creatine kinase. J Steroid Biochem Mol Biol 256:5741–5749.

Shao W, Keeton EK, McDonnell DP, Brown M. 2004. Coactivator AIB1 links estrogen receptor transcriptional activity and stability. Proc Natl Acad Sci USA 101: 11599–11604.

Somjen D, Weisman Y, Harell A, Berger E, Kaye AM. 1989. Direct and sex-specific stimulation by sex steroids of creatine kinase activity and DNA synthesis in rat bone. Proc Natl Acad Sciences USA 86:3361–3363.

Somjen D, Kohen F, Jaffe A, Amir-Zaltzman Y, Knoll E, Stern N. 1998. Effect of gonadal steroids and their antagonists on DNA synthesis in human vascular cells. Hypertension 32:39–45.

Somjen D, Waisman A, Weisman Y, Kaye AM. 2000. "Non-hypercalcemic" analogs of 1α, 25 dihydroxy vitamin D augment the induction of creatine kinase B by estrogen and selective estrogen receptor modulators (SERMS) in osteoblast-like cells and rat skeletal organs. J Steroid Biochem Mol Biol 72:79–88.

Somjen D, Knoll E, Kohen F, Stern N. 2001a. Effects of phytoestrogens on DNA synthesis and creatine kinase activity in vascular cells. Amer J Hypertension 14:1256–1262.

Somjen D, Waisman A, Lee J-K, Posner GH, Kaye AM. 2001b. A non-calcemic analog of 1α, 25 dihydroxy vitamin

D_3 (JKF) upregulates the induction of creatine kinase B by 17β estradiol in osteoblast-like ROS 17/2.8 cells and in rat diaphysis. J Steroid Biochem Mol Biol 77:205–212.

Somjen D, Paller CJ, Gayer B, Kohen F, Knoll E, Stern N. 2004. High glucose blocks estradiol's effects on human vascular cell growth: Differential interaction with estradiol and raloxifene. J Steroid Biochem Mol Biol 88:101–110.

Somjen D, Kohen F, Gayer B, Sharon O, Limor R, Kulik T, Knoll E, Stern N. 2004a. Role of putative membrane receptors in estradiol's effects on human vascular cell growth. Amer J Hyperten 17:462–469.

Somjen D, Katzburg S, Kohen F, Gayer B, Sharon O, Hendel D, Kaye AM. 2005a. Responsiveness to estradiol-17β and to phytoestrogens in primary human osteoblasts is modulated differentially by high glucose concentration. J Steroid Biochem Mol Biol in press.

Somjen D, Kohen F, Lieberherr M, Gayer B, Schejter E, Katzburg S, Limor R, Sharon O, Knoll E, Posner GH, Kaye AM, Stern N. 2005b. 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 93:293–303.

Talukder AH, Gururaj A, Mishra SK, Vadlamudi RK, Kumar R. 2004. Metastasis-associated protein 1 interacts with NRIF3, an estrogen-inducible nuclear receptor coregulator. Mol Cell Biol 24:6581–6591.

Williams JK, Honore EK, Adams DUM. 1997. Contrasting effects of Conjugated estrogens and tamoxifen on dilator responses of atherosclerotic epicardial coronary arteries in nonhuman primates. Circulation 96:1970–1975.

Yamauchi T, Ohuaka K, Takayanagi R, Umeda F, Nawata H. 1990. Enhanced secretion of endothelin 1 by elevated glucose levels from cultured bovine aortic endothelial cells. FEBS Lett 267:16–18.