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17β-Estradiol modulates effects of insulin-induced changes in vascular contractility

INVESTIGACIÓN BÁSICA

17β-Estradiol modulates effects of insulin-induced changes in vascular contractility

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Summary

The protective role of estrogens against peripheral vascular and coronary disease in women is well documented; however, it is not present in diabetic women. Estrogens reduce tension development through non-genomic mechanisms that include changes in calcium concentrations in endothelial and smooth muscle cells, and regulation of nitric oxide synthase (NOS) in endothelial cells. Insulin increases endothelin-1 (ET-1) release from endothelial cells modulating smooth muscle calcium levels and elevating force generated by femoral and coronary arteries. This paper examines whether 17β-estradiol ($E_2\beta$) modulates changes in femoral and coronary artery contractility induced by insulin. Femoral and coronary arteries were obtained from male Wistar rats, placed in isolated tissue baths for *in vitro* studies, perfused with different solutions, and the contractile response to KCl 40 mmol/L was measured. Insulin increased arterial contraction induced by KCl. This increase was not present when the endothelium was removed. In the presence of $E_2\beta$, we observed a dose dependent reduction in the tension developed and this effect disappeared when the endothelium was removed. The insulin-induced contraction was significantly reduced in presence of $E_2\beta$. These data indicate that the effect of insulin on femoral and

Resumen

El 17β-estradiol modula los efectos de los cambios de la contractilidad vascular producidos por la insulina

Los estrógenos protegen a la mujer contra enfermedades vasculares periféricas y centrales; sin embargo, su papel se pierde con la diabetes. Los estrógenos reducen la tensión en las arterias mediante cambios en el calcio intracelular en células endoteliales y musculares lisas y la regulación de la óxido nítrico sintasa en células endoteliales. La insulina incrementa la liberación de endotelina-1 (ET-1) en células endoteliales aumentando la fuerza generada por las arterias. En este estudio se examina si el 17β-estradiol ($E_2\beta$) modula los cambios en la contractilidad inducidos por insulina en las arterias femorales y coronarias. Las arterias se obtuvieron de ratas Wistar macho y se colocaron en cámaras para tejido aislado para perfundirse *in vitro* con distintas concentraciones de insulina y estrógenos estimulando la contracción con KCl 40 mmol/L. La insulina elevó la fuerza de la contracción inducida por KCl. Este incremento desapareció cuando se eliminó el endotelio. El $E_2\beta$ disminuyó la tensión desarrollada por las arterias conforme se aumentó la dosis y el efecto desapareció al quitar el endotelio. El incremento en la tensión

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coronary vascular contractility is modulated by $E_2\beta$.

por insulina disminuyó con $E_2\beta$. En conclusión el efecto de la insulina sobre las arterias femorales y coronarias se encuentra modulado por el $E_2\beta$.
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Key words: Insulin. Estrogen. Endothelium. Coronary arteries. Femoral arteries.
Palabras clave: Insulina. Estrógenos. Endotelio. Arterias coronarias. Arterias femorales.

Introduction

The protective role of estrogens has been well documented and several reports have demonstrated that premenopausal women have lower incidence of peripheral and coronary vascular disease than postmenopausal women.[1,2] However, in diabetic women the normal premenopausal gender related difference in the prevalence of cardiovascular disease is lost.[3,4] Although the mechanisms of action of estrogens and insulin on vascular smooth muscle cells and on vascular endothelium have been extensively studied, they are still not completely understood and probably many interacting mechanisms may be involved in the effects of these hormones.

Steroids exert some of their effects on target cells by entering them, binding to high affinity cytoplasmic or nuclear receptors to constitute the steroid-receptor complex that interacts specifically with DNA. This complex regulates gene transcription and production of specific proteins that modify cell function.[5] However, this mechanism does not account for all of the cellular effects of steroid hormones, signaling the existence of nongenomic mechanisms, particularly for the rapid, transient effects, which seem to be independent of hormone interaction with the cell nucleus.[6] One of the alternative mechanisms of action proposed in endothelial cells is an increase in intracellular calcium $[Ca^{2+}]i$ which is necessary for NO synthesis.[7-10] In addition, cytosolic and plasma membrane estrogen receptors on vascular smooth muscles cells have been found and are known to block calcium entry through L-type calcium channels, reducing $[Ca^{2+}]i$ and inducing relaxation.[11]

In a previous paper we demonstrated that insulin increases force generated by femoral and coronary arteries and that these effects are mediated by ET-1.[12] ET-1 induces elevation of $[Ca^{2+}]i$ concentration in vascular smooth muscle cells[11] and increases vascular tone. Therefore, the aim of the present report was to examine whether $E_2\beta$ modulates changes of femoral and coronary artery contraction induced by insulin through rapid mechanisms, particularly affecting endothelial cell function rather than smooth muscle contraction.

Methods

Vascular tissue preparation and tension recording

Male Wistar rats weighing 250 to 350 g were used. The animals were killed by cervical dislocation, and the heart or femoral arteries were dissected immediately and kept in an oxygenated standard Tyrode solution of the following composition (mM): 136.9 NaCl, 5.4 KCl, 1 $CaCl_2$, 1.05 $MgCl_2$, 11.9 $NaHCO_3$, 0.33 NaH_2PO_4, and 5.5 glucose. Under a dissecting microscope, the femoral vessels were cleaned of surrounding tissue, avoiding damage to the endothelium. Arterial segments of 5 mm were cut, and two 250-µm-diameter S-shaped silver hooks (Medwire) were inserted into the lumen to measure the tension developed transversally. For experiments in coronary arteries, the first wire string was inserted into the left coronary artery in the intact heart. The wall of the vessel was lifted to insert the second wire. A coronary artery segment of 2 to 3 mm was then dissected from the ventricle wall and partially cleaned from cardiac muscle. One of the silver hooks was fixed to the bottom of an organ chamber, and the other was attached to an isometric force-displacement transducer (FT03, Grass Medical Instruments). The transducer was connected to a Grass polygraph model 79 D. The optimal basal passive tensions for rings of both types of vessels were previously found under our experimental conditions[12] and applied: 500 mg for femoral arteries and 250 mg for coronary arteries. Basal passive tension for coronary arteries was smaller than the one applied to femoral arteries since these vessels are normally perfused during diastole in which arterial pressure is smaller than in arteries perfused during systole. The experiments were also done in arteries in which the endothelium was removed by gently rubbing the vessel with the silver strings against a wet piece of cloth. The

integrity or absence of the endothelium was proved by testing the response to acetylcholine (10^{-6} M) at the end of the experiment.

Experimental protocol

The vessels were perfused with standard Tyrode solution bubbled with a 95% $O_{2,}$ 5%_CO_2 gas mixture. The chamber was continually perfused with warm (37°C) solution. The rings were allowed to equilibrate for 15 min. Contractions were induced by a Tyrode solution containing 40 mmol/L KCl; this concentration was chosen after concentration-response curves had been obtained since it produced submaximal responses (data not shown). After recording control-developed tension, the arteries were washed with normal Tyrode solution and were allowed to return to their basal tension level. The procedure was repeated, and the mean value of the force developed was taken as a 100% response.

After this, the arteries were subjected to one of the following situations in separate experiments repeating the stimulation with KCl to test the contractile response: 1. Arteries were perfused with different doses (150, 300, 600 pmol/L) of insulin (Eli Lilly & Company). These concentrations were chosen to include fasting (150 pmol/L) and posprandial (300 pmol/L) normal concentrations. 2. Arteries were perfused with different doses (10 to 10,000 pmol/L) of $E_2\beta$. These concentrations were chosen to include estrus (350-450 pmol/L) and diestrum (80-120 pmol/L) normal concentrations in female rats from the same species. 3. Arteries were perfused with insulin (300 pmol/L) in the presence of $E_2\beta$ (100 pmol/L). 4. At the end of each experiment, arteries were bathed again with standard Tyrode solution and contracted with KCl to verify that there were no important effects secondary to the incubation time or to repeated exposures to KCl.

Statistical analysis

Values are expressed as percentages; basal contraction was considered as 100% and the percentage in each experiment calculated. Mean and standard errors of at least six different arteries were calculated. Statistical significance of the differences was analyzed using Students *t test* to compare femoral and coronary arteries under the same experimental situation and ANOVA followed by Newman-Keuls test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

To investigate the role of $E_2\beta$, as a modulator of insulin effect, we compared the effects of $E_2\beta$ on the response to KCL in the presence and absence of insulin in femoral and coronary arteries, with and without endothelium. No effects on passive basal tension were observed as the vessels were perfused with solutions containing different insulin or $E_2\beta$ concentrations. Nevertheless, when the arteries were contracted by the addition of KCl, tension development varied under the different experimental conditions. Basal contraction force in femoral and coronary arteries bathed with normal Tyrode (without insulin or $E_2\beta$) was 28 ± 15 and 134 ± 18 mg respectively and was considered as 100% in all experiments.

Insulin-induced increases in vascular contractility. Endothelial-mediation in the effects

We found a dose-dependent increase in force generated to KCl by femoral and coronary arteries with standard Tyrode solution and in the presence of different insulin concentrations. The maximal force increase for femoral arteries was found at 300 pmol/L, reaching 175.48 ± 12.20% (n = 10), whereas for coronary arteries the largest increase was found with a lower dose of 150 pmol/L and the force reached only 130.42 ± 7.8% *(Fig. 1)*.

Mediation by the endothelium in the insulin effects on femoral and coronary arteries was tested after removing it. We observed that, when the endothelium was removed, KCl-induced contractions in the presence of insulin in femoral and coronary arteries were not significantly modified from the control contractions and therefore the insulin effect disappeared. *(Fig. 2A)*.

Effects of different $E_2\beta$ concentrations on vascular contractility

When femoral and coronary arteries were bathed with normal Tyrode solution, in the presence of $E_2\beta$, we observed a dose-dependent reduction in tension developed to KCl by the arteries. In both arteries, a significant decrease of approximately 35% of the control contraction (to 68.37 ± 4.14% in femoral and to 66.70 ± 4.87 in coronary arteries) was reached with the lower dose of 10 pmol/L. The minimal response was reached in both arteries with the higher dose of 10,000 pmol/L representing a decrease of approximately 75%

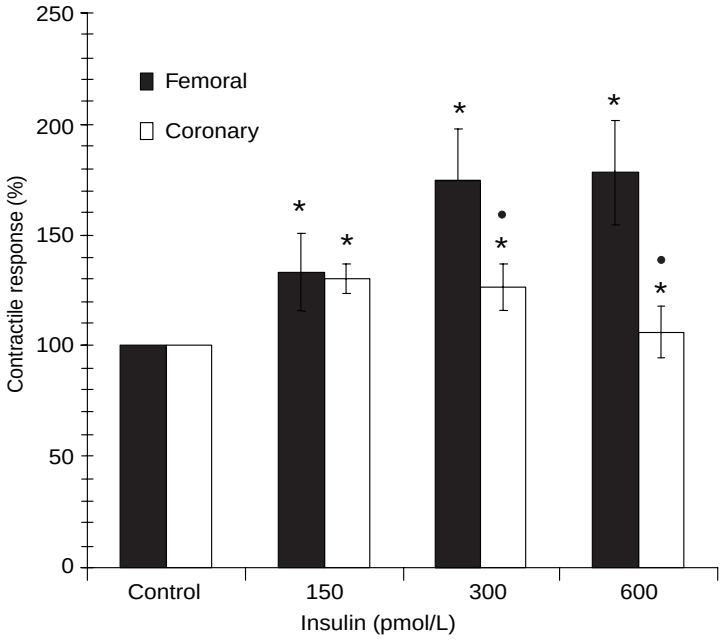

Fig. 1. Effects of different insulin concentrations on contractile response to KCl of femoral and coronary arteries. Values are means ± SEM, n = 6 *P < 0.05 compared to values without insulin. •P < 0.05 when comparing femoral against coronary arteries.

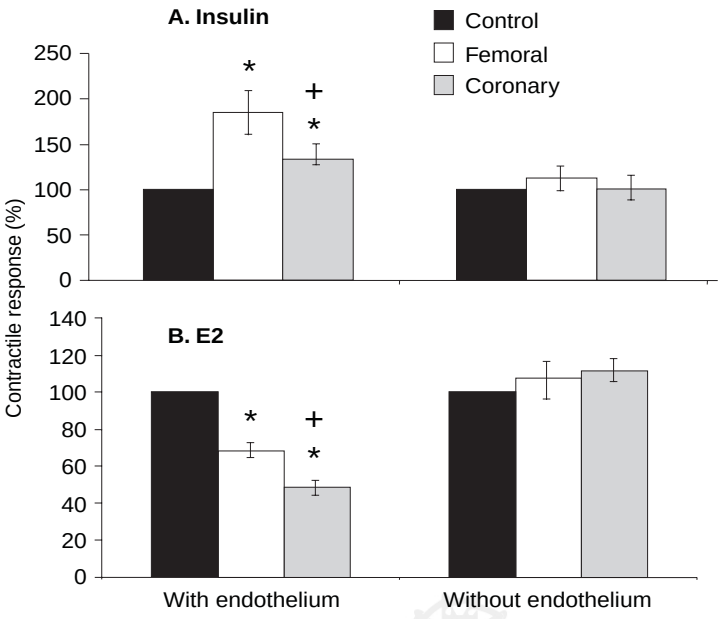

Fig. 2. (A) Effects of the presence and absence of endothelium on the contractile response to KCl in the presence of insulin (300 pmol/L) in femoral and coronary arteries. (B) Effects of the presence and absence of endothelium on the contractile response to KCl in the presence of $E_2\beta$ (100 pmol/L) in femoral and coronary arteries. Values are means ± SEM, n = 6. *P < 0.05 when comparing arteries against the control response with and without endothelium, + P < 0.05 when comparing femoral against coronary arteries.

of the control contraction (to 34.28 ± 3.17% in femoral and to 35.9 ± 4.31% in coronary arteries) *(Fig. 3)*. Endothelial-mediation of the effect of $E_2\beta$ was also tested. When the endothelium was removed in femoral and coronary arteries, the decrease in tension observed in vessels perfused with $E_2\beta$ (100 pmol/L) disappeared and contractility returned to the basal level *(Fig. 2B)*.

$E_2\beta$ modulator effect of insulin-induced changes in vascular contractility

$E_2\beta$ mediation of the insulin effects on femoral and coronary arteries was tested using a solution with insulin (300 pmol/L) and $E_2\beta$ (100 pmol/L). When both hormones were applied simultaneously, only a slight non-significant increase on vascular tension was observed (to 119 ± 11.7% in femoral and 113 ± 7.22% in coronary arteries). Neither the increase in tension induced by insulin to 175.48 ± 12.20% and 130.42 ± 7.8% (n = 10) in femoral and coronary arteries respectively nor the decrease induced by estrogen of 35% (to 65 ± 11.86%) in femoral and of 24% (to 76.92 ± 2.93%) in coronary arteries were observed. These data indicate that the $E_2\beta$ effects are strong enough to block the insulin-induced increase in vascular tension *(Fig. 4)*.

Discussion

The incidence of cardiovascular disease with age is increasing, and elderly women constitute an important component of the aging population. Elderly women also have a relatively high incidence of diabetes.[13] Although cardiovascular disease is less common in premenopausal women that in men, this difference begins to disappear after the onset of menopause, presumably related to decreased levels of female sex hormones.[1,4] Diabetes mellitus removes the normal premenopausal gender-related differences in the prevalence of cardiovascular disease by mechanisms that are not clearly defined.[3,4] Furthermore, the risk of death from cardiovascular disease in women with diabetes is more than 3 times that of nondiabetic women.[13] Many factors contribute to the increase in cardiovascular disease in diabetic women as well as men. These include endothelial dysfunction, increased vascular oxidative stress, and abnormalities of platelet function, coagulation, fibrinolysis, and lipoproteins.[14] The aim of the present study was to examine whether $E_2\beta$ modulates the increases in femoral and coronary artery contractility induced by insulin,

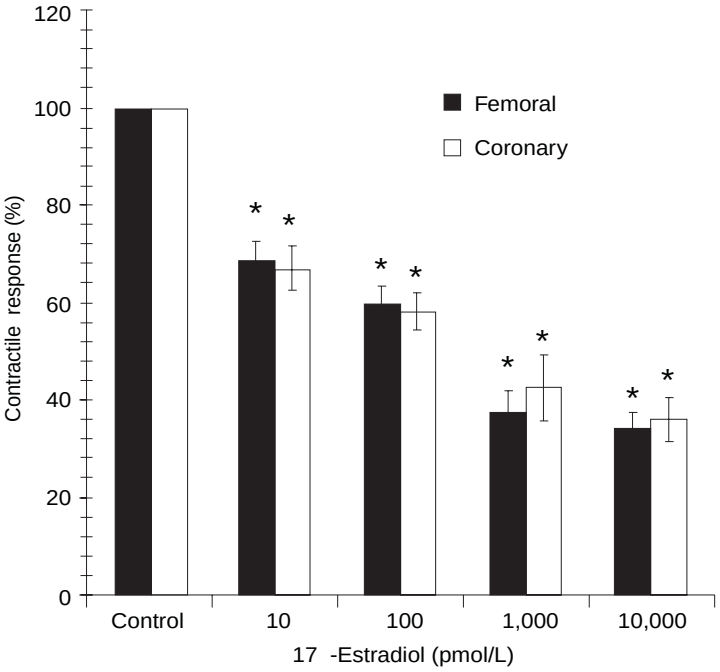

Fig. 3. Effects of different $E_2\beta$ concentrations on contractile response to KCl of femoral and coronary arteries. Values are means ± SEM, n = 6 *P < 0.05 compared to values without $E_2\beta$. There were no significant differences between femoral and coronary arteries.

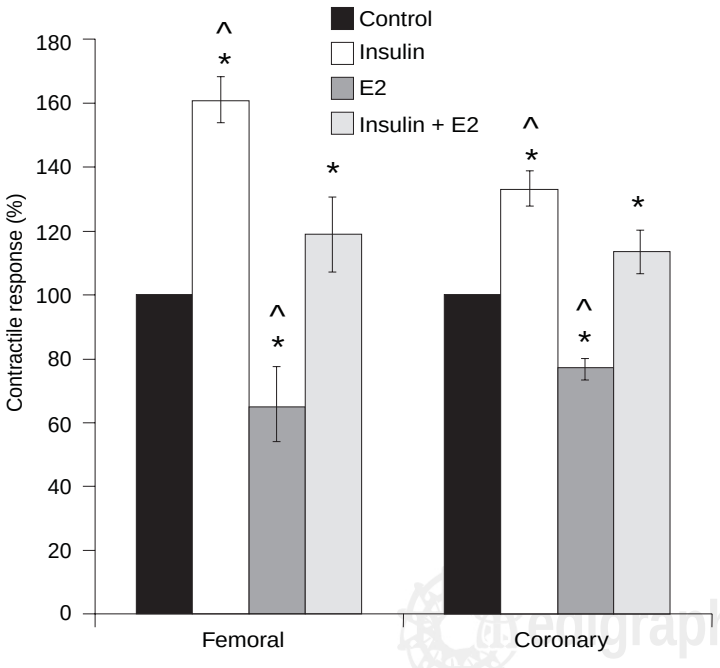

Fig. 4. Compensation of the insulin response (300 pmol/L) by the presence of $E_2\beta$ (100 pmol/L) in femoral and coronary arteries. Values are means ± SEM, n = 6. *P <0.05 when comparing the effects of the hormones to the control. ^ P < 0.05 when comparing the response with insulin and $E_2\beta$ against the contraction to each of the hormones independently.

that we have previously demonstrated,[12] through rapid mechanisms affecting endothelial cells.

In our experiments, insulin increased the force generated by femoral and coronary arteries and these effects were larger as insulin concentrations rose. Although it is clear that diabetes mellitus is associated with an increase in vascular complications despite tight control of blood glucose, there is a controversy on the role of insulin on vascular contractility. Insulin has been reported to influence membrane components in different cells and situations that may increase or decrease intracellular calcium levels and contribute to the contradicting tension development changes found in vascular structures.[8,15-18]. Insulin has also been reported to modify endothelial cell secretion inducing both vasoconstriction and vasodilatation.[19-22] It reduces α-adrenergic induced vasoconstriction by stimulating the production of endothelial nitric oxide through a pertussis toxinsensitive mechanism.[23,24] However, in accordance with our data, other studies have shown a correlation between high insulin levels and hypertension.[3,8,14,19] In bovine and porcine endothelial cells, insulin stimulates endothelin production and secretion and increases ET-1 gene expression.[20] We have previously reported an increased femoral contraction with insulin that is mediated by endothelin.[12]

Several possible mechanisms of action of estrogens on human tissues have been proposed to explain the multiple observational clinical and experimental studies suggesting a marked reduction in risk of peripheral and coronary disease associated with postmenopausal estrogen use.[4,25,26] In the intact organisms, changes in plasma lipoprotein levels, fibrinogen, antithrombin III, angiotensin type 1 receptor regulation, and antioxidant action have been proposed.[27,28] Estrogens have also been found to have vascular local effects and their acute administration relaxes vascular smooth muscle, causing a reduction in vascular resistance and increased blood flow.[29-31] These reports agree with our results, in which we found a decreased force of contraction as estrogen concentrations rose.

Several studies have proposed the classical signaling pathway for the genomic effect of estrogens. In this pathway, the estrogen receptor translocates to the nucleus and binds directly to response elements to regulate gene transcription.[5] In addition, several non-classical pathways have been described to explain the rapid changes in-

duced by estrogens, which are clearly not compatible with the genomic mechanisms.[6,9,32] It is not clear which of these estrogen mechanisms predominate in each of the different cell types. One of the genomic mechanisms described for $E_2\beta$ that may explain in part our results in vessels is the stimulation of NOS-III gene expression in the rat uterus.[33] Some of the non-genomic mechanisms that might help explain our results include the depolarization of the plasma membrane, in pancreatic β-cells eliciting electrical activity and intracellular calcium signals.[34] Vascular smooth muscle responds to $E_2\beta$ reducing $[Ca^{2+}]i$[35,36] by blocking L-type Ca^{2+} channels.[9,37,38] Although our experiments with estrogens could be explained by this mechanism, the fact that relaxation disappeared in the absence of endothelium suggests that estrogens modify endothelial function rather than smooth muscle cell contractility. Our results suggest that an impairment of the endothelial cell function may be responsible for the effects of estrogens on isolated vessels and for protection from vascular disease. Endothelial cells have estrogen receptors whereas subendothelial cells do not.[39] Estrogens activate endothelial NOS, which is responsible for the relaxing effect by mechanisms of action that remain to be elucidated.[31,40,41] It could be possible that the mechanism involves the increase in $[Ca^{2+}]i$ level, which induces expression and/or activity of endothelial NOS and NO release.[42,43] Other vasodilator substances released by the endothelium such as prostacyclin, endothelium derived hyperpolarizing factor, epoxy-eicosatrienoic acids, and adenosine might also be involved in the relaxing effects of $E_2\beta$.[37]

Our results show that estrogens modulate the insulin effect only when endothelial cells are present. Therefore, $E_2\beta$ counterbalances the vasoconstrictor effect of insulin by altering endothelial function. Estrogens have not been reported to modify or block the regulatory mechanisms of action induced by insulin and apparently they use independent mechanisms to induce relaxation. When both hormones are present at the same time, their effects are compensated by the antagonistic mechanism of the other hormone. The release of both vasoconstrictor and dilator substances is increased, having opposite effects and therefore neither the contractile effect of insulin nor the dilator effect of $E_2\beta$ predominate. This might be one of the reasons why diabetic women are not protected against coronary and peripheral vascular disease during the reproductive age. Furthermore, it has been shown that male diabetic rats exhibit the deleterious effect of the disease and that chronic $E_2\beta$ treatment reduces injury in male diabetic rats, providing vascular protection.[44]

Our findings support the idea that endothelial dysfunction may represent the pathogenic mechanism in disease states associated with altered blood flow, such as diabetes, hypertension,[45] and cardiovascular disease. Vascular endothelial cells have the ability to modulate local vascular tone by releasing relaxing factors or vasoconstrictor peptides. We conclude that $E_2\beta$, modulates the increases in femoral and coronary artery contractility induced by insulin, acting upon the endothelial cells. These results help explain the lost protective role of estrogens against peripheral and coronary vascular disease in diabetic women.

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References

1. Nabulsi A, Folsom AR, White A, Patsch W, Heiss G, Wu KK, et al: *Association of hormone-replacement therapy with various cardiovascular risk factors in post-menopausal women*. N Engl J Med 1993; 328: 1069-1075.
2. Kafonek SD: *Postmenopausal hormone replacement therapy and cardiovascular risk reduction.* Drugs 1994; 47: 16-24.
3. Kannel WB: *Risk stratification in hypertension: new insights from the Framingham Study*. Am J Hypertens 2000; 13: 3S-10S.
4. Barret-Connor E, Grady D: *Hormone replacement therapy, heart disease, and other considerations*. Annu Rev Public Health 1998; 19: 55-72.
5. Carson-Jurica MA, Schrader WT, O'Malley BW: *Steroid receptor family: structure and functions*. Endocr Rev 1990; 11: 201-220.
6. Nabekura J, Oomura Y, Minami T, Mizuno Y, Fukuda A: *Mechanism of the rapid effect of 17 beta-estradiol on medial amygdala neurons*. Science 1986; 233: 226-228.
7. Evans R: *The steroid and thyroid hormone re-*

ceptor superfamily. Science 1988; 240: 889-895.
8. Epstein M, Sowers JR: *Diabetes mellitus and hypertension*. Hypertension 1992; 19: 403-418.
9. Rubio-Gayosso I, Sierra-Ramirez A, García-Vazquez A, Martinez-Martinez A, Munoz-García O, Morato T, et al: *17b-Estradiol increases intracellular calcium concentration through a short-term and nongenomic mechanism in rat vascular endothelium in culture*. J Cardiovasc Pharmacol 2000; 36: 196-202.
10. Rodriguez J, Garcia de Boto MJ, Hidalgo A: *Mechanisms involved in the relaxant effect of estrogens on rat aorta strips*. Life Sci 1996; 58: 607-615.
11. Prakash YS, Togaibayeva A, Kannan MS, Miller VM, Fitzpatrick LA, Sieck GL: *Estrogen increases Ca^{2+} efflux from female porcine coronary arterial smooth muscle*. Am J Physiol 1999; 276: H926-H934.
12. Nava P, Masso F, Collados T, Guarner V: *Endothelin mediation of insulin and glucose-induced changes in vascular contractility*. Hypertension 1997; 30: 825-829.
13. Kannel WB: *Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study*. Am Heart J 1987; 114: 413-419.
14. Sowers JR, Epstein M, Frohlich ED: *Diabetes, hypertension, and cardiovascular disease: an update*. Hypertension 2001; 37: 1053-1059.
15. Levy J, Zemel MB, Sowers JR: *Role of cellular calcium metabolism in abnormal glucose metabolism and diabetic hypertension*. Am J Med 1989; 87: 7S-16S.
16. Resnick L: *Hypertension and abnormal glucose homeostasis*. Am J Med 1989; 87: 17S-22S.
17. Barbagallo M, Gupta RK, Dominguez LJ, Resnick LM: *Cellular ionic alterations with age: relation to hypertension and diabetes*. J Am Geriatr Soc 2000; 48: 1111-1116.
18. Reaven GM, Hoffman BB: *Hypertension as a disease of carbohydrate and lipoprotein metabolism*. Am J Med 1989; 87: 2S-6S.
19. Park JY, Takahara N, Gabriele A, Chou E, Naruse K Suzuma K, et al: *Induction of endothelin-1 expression by glucose: an effect of protein kinase C activation*. Diabetes 2000; 49: 1239-1248.
20. Oliver FJ, de la Rubia G, Feener EP, Lee ME, Loeken MP, Shiba T, et al: *Stimulation of endothelin-1 gene expression by insulin in endothelial cells*. J Biol Chem 1991; 266: 23251-23256.
21. Piatti PM, Monti LD, Conti M, Baruffaldi L, Galli L, Phan CV, et al: *Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans*. Diabetes 1996; 45: 316-321.
22. Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, et al: *Regulation of endothelial constitutive nitric oxidesynthase gene expression in endothelial cells and in vivo. A specific vascular action of insulin*. Circulation 2000; 101: 676-681.
23. Kahn AM, Husid A, Odebunmi T, Allen JC, Seidel CL, Song T: *Insulin inhibits vascular smooth muscle contraction at site distal to calcium concentration*. Am J Physiol 1998; 5: E885-E892.
24. Lembo G, Iaccarino G, Vecchione C, Barbato E, Morisco C, Monti F, et al: *Insulin enhances endothelial adrenergic vasorelaxation by a pertussis toxin mechanism*. Hypertension 1997: 1128-1134.
25. Grodstein F, Manson JE, Stampfer MJ: *Postmenopausal hormone use and secondary prevention of coronary events in the nurses' health study. A prospective, observational study*. Ann Intern Med 2001; 135: 1-8.
26. Binder EF, Williams DB, Schechtman KB, Jeffe DB, Kohrt WM: *Effects of hormone replacement therapy on serum lipids in elderly women. a randomized, placebo-controlled trial*. Ann Intern Med 2001; 134: 754-760.
27. Nickenig G, Wassmann S, Bohm M: *Regulation of the angiotensin AT1 receptor by hypercholesterolaemia*. Diabetes Obes Metab 2000; 2: 223-228.
28. Wassmann S, Laufs U, Baumer AT, Muller K, Ahlbory K, Linz W, et al: *HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolemic hypertension via reduced production of reactive oxygen species*. Hypertension 2001; 37: 1450-1457.
29. Gilligan DM, Badar DM, Panza JA, Ouyyumi AA, Cannon RO 3rd: *Acute vascular effects of estrogen in postmenopausal women*. Circulation 1994; 90: 786-791.
30. Zacharia LC, Jackson EK, Gillespie DG, Dubey RK: *Increased 2-methoxyestradiol production in human coronary versus aortic vascular cells*. Hypertension 2001; 37: 658-662.
31. Rupnow HL, Phernetton TM, Shaw CE, Modrick ML, Bird IM, Magness RR: *Endothelial vasodilator production by uterine and systemic arteries. VII. Estrogen and progesterone effects on eNOS.* Am J Physiol (Heart Circ Physiol) 2001; 280: H1699-705.
32. Kitazawa T, Hamada K, Kitazawa K, Gaznabi AK: *Non-genomic mechanism of 17 beta-oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle*. J Physiol 1997; 499: 497-511.
33. Yallampalli C, Dong Y-L: *Estradiol-17ß inhibits nitric oxide synthase (NOS)-II and stimulates NOS-III gene statement in the rat uterus*. Biol Reprod 2000; 63: 34-41.
34. Ropero AB, Fuentes E, Rovira JM, Ripoll C, Soria B, Nadal A: *Non-genomic actions of 17beta-oestradiol in mouse pancreatic beta-cells are mediated by a cGMP-dependent protein kinase*. J Physiol 1999; 521: 397-407.
35. Morley P, Whitfield JF, Vanderhyden BC, Vanderhyden BC, Tsang BK, Schwartz JL: *A new nongenomic estrogen action: the rapid release of intracellular calcium*. Endocrinology 1992; 131: 1305-1312.

36. Raddino R, Manca C, Poli E, Bolognesi R, Visioli O: *Effects of 17 beta-estradiol on the isolated rabbit heart*. Arch Int Pharmacodyn Ther 1986; 281: 57-65.
37. Ruehlmann DO, Mann GE: *Rapid non-genomic vasodilator actions of oestrogens and sex steroids*. Curr Med Chem 2000; 7: 533-541.
38. Meyer R, Linz KW, Surges R, Meinardus S, Vees J, Hoffmann A, et al: *Rapid modulation of L-type calcium current by acutely applied oestrogens in isolated cardiac myocytes from human, guinea-pig and rat*. Exp Physiol 1998; 83: 305-321.
39. Colburn P, Buonassisi V: *Estrogen-binding sites in endothelial cell cultures*. Science 1978; 201: 817-819.
40. Tamura K, Yamaguchi K, Kogo H: *17Beta-estradiol inhibits ovariectomy-induced expression of inducible nitric oxide synthase in rat aorta in vivo*. Life Sci 2000; 66: PL 259-264.
41. Gonzales RJ, Walker BR, Kanagy NL: *17beta-estradiol increases nitric oxide-dependent dilation in rat pulmonary arteries and thoracic aorta*. Am J Physiol Lung Cell Mol Physiol 2001; 280: L555-564.
42. Gorodeski GI: *Calcium regulates estrogen increase in permeability of cultured CaSki epithelium by eNOS-dependent mechanism*. Am J Physiol Cell Physiol 2000; 279: 495-505.
43. Yang S, Bae L, Zhang LJ: *Estrogen increases eNOS and NOx release in human coronary artery endothelium.* Cardiovasc Pharmacol 2000; 36: 242-247.
44. Toung TK, Hurn PD, Traystman RJ, Sieber FE: *Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type 1 diabetes mellitus*. Stroke 2000; 31: 2701-2706.
45. Haefliger J, Flammer TF, Luscher: *Nitric oxide and endothelin-1 are important regulators of human ophthalmic artery*. Invest Ophthalmol Visual Sci 1992; 33: 2340-2343.