ELSEVIER

International Journal of Cardiology 107 (2006) 254 – 259

International Journal of Cardiology

www.elsevier.com/locate/ijcard

# Effect of estradiol 17β upon coronary artery vasoconstrictor response to methylergometrine maleate in female menopausal patients

Giuseppe M.C. Rosano[a,*,1], Peter Collins[b,c], Otavio Gerbara[a], Imad Sheiban[e], Antonello Silvestri[a], Mauricio Wajngarten[d], Josè A. Ramires[d], Massimo Fini[a], Giuseppe Mercuro[b,c]

[a]*Department of Medical Sciences, IRCCS San Raffaele Hospital, Tosinvest Sanità, via della Pisana 235, 00164 Roma, Italy*
[b]*National Heart and Lung Institute, London, U.K.*
[c]*Department of Cardiovascular Sciences, University of Cagliari, Cagliari, Italy*
[d]*Istituto do Coracao, Hospital das Clinicas, University of Sao Paulo, Sao Paulo, Brazil*
[e]*Department of Cardiology, University of Torino, Torino, Italy*

Received 4 January 2005; received in revised form 11 March 2005; accepted 12 March 2005
Available online 10 May 2005

**Abstract**

*Background:* Estrogens produce several beneficial effects upon the cardiovascular system. Amongst these, an endothelium-independent effect has been convincingly demonstrated only in vitro, while there is no evidence for such an effect in vivo. The aim of the present study was to evaluate the effect of acute administration of estradiol 17β upon coronary artery reactivity to methylergometrine in 16 menopausal patients with coronary artery disease.
*Methods:* Sixteen menopausal patients underwent coronary angiography at rest and after incremental doses of methylergometrine (intracoronary 2, 10, 30 μg) before and 20 min after either intracoronary estradiol 17β (20 ng/mL at 1 mL/min for 20 min; 8 patients) or placebo (Dextrose 5%, 1 mL/min; 8 patients).
*Results and conclusions:* No significant differences were observed in baseline coronary artery diameter or area between the 2 groups. No significant differences in the degree of coronary artery constriction were observed after either estradiol 17β or placebo at submaximal doses of methylergometrine. However, the degree of coronary artery constriction after maximal doses of methylergometrine was significantly attenuated by estradiol 17β compared to placebo (change in diameter: $-0.9\pm4.5\%$ vs. $-19\pm6\%$, $p<0.001$; change in area: $-3.2\pm9\%$ vs. $-32.2\pm10\%$, $p<0.001$). Estradiol 17β reduces coronary artery constriction following methylergometrine administration in menopausal patients with coronary artery disease. This effect may be related to the calcium-antagonist properties of the ovarian hormone.
*Condensed Abstract:* The effect of acute administration of estradiol 17β upon coronary artery reactivity to methylergometrine was evaluated by coronary angiography in 16menopausal women with CAD. The investigation was performed at rest and after incremental dose of methylergometrine before and 20 min after either intracoronary estradiol 17β or placebo. Significant differences were not observed either in the baseline coronary artery diameter or the area between the 2groups, or in the degree of coronary artery constriction after either estradiol 17β or placebo at submaximal doses of methylergometrine. Coronary artery constriction after maximal doses of methylergometrine resulted attenuated by estradiol 17β for its calcium-antagonist properties.


*Keywords:* Women; Estrogen; Coronary artery disease

## 1. Introduction

Despite recent data from the WHI and the Heart and Estrogen/Progestin Replacement Study, which failed to demonstrate a protective effect of estrogens upon cardiovascular events, several studies have shown that current

* Corresponding author. Tel.: +39 6 660581; fax: +39 6 52244512.
*E-mail address:* giuseppe.rosano@sanraffaele.it (G.M.C. Rosano).
[1] Dr. Rosano was supported by an educational grant of the Consiglio Nazionale delle Ricerche-National Research Council–Rome (Italy).

0167-5273/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijcard.2005.03.030

estrogen replacement therapy reduces the risk of acute ischemic syndromes in hormone users as compared to non users [1–3]. In the past years, great importance has been attributed to the metabolic effects of the ovarian hormones. Indeed, estrogens reduce total and LDL cholesterol, increase HDL cholesterol [4–6], improve carbohydrate metabolism and insulin sensitivity [7,8], positively influence the coagulation profile [9], and are involved in the formation and development of the atherosclerotic plaque [10–12]. Recent evidence suggests that most of the cardiovascular protective effects might be dependent on a direct action of the ovarian hormones upon cardiovascular functions.

Animal studies have shown that estrogens are vasoactive substances acting in almost all vascular districts [13–18]. In vitro studies have shown that estradiol 17β has a relaxing effect upon precontracted rabbit and human coronary artery rings [14–16] and that estradiol 17β reveals calcium antagonistic properties [17,18]. In vivo studies have demonstrated that estrogens potentiate the endothelium-dependent vasodilator response to acetylcholine in animals [12] and in humans [19–21]. Clinical studies have shown that acute administration of sublingual estradiol 17β improves exercise-induced myocardial ischemia in menopausal patients with coronary arteries [22] and reduces peripheral vascular resistances [23,24]. Since coronary artery tone plays an important role in the pathogenesis of cardiac ischemic syndromes, an effect of estradiol 17β upon coronary artery reactivity may be of importance in the cardioprotective effect of the hormone.

The aim of the present study was to evaluate the effect of acute administration of estradiol 17β upon methylergometrine-induced vasoconstriction in menopausal patients with coronary artery disease.

## 2. Methods

### 2.1. Patients

The study population included 25 female menopausal patients who referred for coronary angiography with angiographically proven coronary artery disease (coronary artery diameter stenosis $\geq$70%) and evidence of being in menopause. Menopause was defined as the absence of menstrual cycles from at least 1 year together with plasma levels of estradiol 17β < 100 pmol/L and follicle stimulating hormone >40 IU/L. Patients taking estrogen replacement therapy, or those with severe hypertension (systolic blood pressure >180 mm Hg and/or diastolic blood pressure >110 mm Hg), recent (<3 months) acute myocardial infarction, unstable angina, heart failure (III or IV NYHA functional classes), clinical suggestion of Prinzmetal's variant angina or primary valvular heart disease were excluded from the study. The study protocol was approved by the local Ethical Committee and all patients gave written informed consent before being included into the study.

### 2.2. Protocol

All patients were studied at the time of diagnostic cardiac catheterization after an overnight fast and in pharmacological wash-out. All cardiac active medications were stopped for at least five half lives before the study, and patients were asked not to smoke or take caffeine-containing drinks in the 24 h prior to the study. After diagnostic cardiac catheterization performed using the Judkins technique, incremental doses of methylergometrine maleate (2, 10, 30 μg) were administered through the diagnostic catheter into a not stenosed coronary artery at 5 min intervals. When the study was performed into the right coronary artery, a temporary pacing lead was inserted into the right ventricle through the right brachial or femoral vein. Coronary arteriograms were obtained using the projections that better visualised the coronary artery under study at the end of each stage of the infusion protocol. If no focal spasm requiring the administration of intracoronary nitrates was detected, patients were randomised to receive, through the coronary catheter, either 400 ng estradiol 17β (20 ng/mL solution) or dextrose (5% solution containing 0.6% ethanol). Both infusions were performed at an infusion rate of 1 mL/min. The administration modality of estradiol 17β was selected according to previous reports, which suggested the attainment of a steady state with venous estradiol 17β concentrations of 300 pg/Ml [20]. Coronary arteriograms were obtained at the end of the drug infusion; at that time, the infusion of methylergometrine maleate was repeated. Sodium nitroprusside 100 μg was administered at the end of this second set of infusions. Arterial blood pressure and electrocardiogram were continuously monitored and recorded at the end of each experimental interval. Blood samples were collected in order to evaluate the plasma levels of estradiol 17β before and at the end of the study session.

### 2.3. Measurement of coronary artery diameters

Coronary arteriograms were analysed in blind by two independent experienced investigators (P.P., I.S.) using a computerized device (Medis Inc.). Major coronary arteries were divided in three parts, using the American Heart Association classification [25]. For the purpose of the study, only angiographically normal segments located in proximal, medial, and distal locations were analysed using a computer assisted edge detection system. To quantify changes in coronary artery diameters, we used the percent vasoconstrictive response to methylergometrine maleate, before and after administration of the study drugs, calculated as follows:

(i) *constriction by methylergometrine maleate*: [(Control diameter − diameter after methylergometrine maleate)/Control diameter] × 100.

(ii) *constriction by methylergometrine maleate after study drug*: [(Control diameter after study-drug − diameter after methylergometrine maleate)/Control diameter after study-drug] × 100.

Table 1
Clinical and angiographic features of patients allocated to estradiol 17β or placebo

| | Estradiol 17β, $n=8$ | Placebo, $N=8$ |
|---|---|---|
| Mean age | 64±4 | 64±5 |
| Years from menopause | 11±3 | 12±4 |
| *Risk factors for CAD* | | |
| Family history | 5 | 4 |
| Hypertension | 2 | 3 |
| Diabetes | 0 | 1 |
| Cholesterol>200 mg/dl | 6 | 5 |
| Cigarette smoking | 2 | 1 |
| *Coronary artery disease* | | |
| 1 vessel disease | 4 | 5 |
| 2 vessel disease | 4 | 3 |

### 2.4. Statistical analysis

Data are expressed as mean±standard deviation, or as percentages, where appropriate. Wilcoxon test for paired data was used to compare measurements of coronary artery diameters at each dose of methylergometrine maleate, before and after the administration of estradiol 17β or ethanol control. Wilcoxon test for unpaired data was used to compare measurements of coronary artery diameters at baseline, after the administration of the study drug, and after sodium nitroprusside, between the two patient groups. A $p$-value <0.05 was considered significant.

## 3. Results

### 3.1. Study patients

Clinical and angiographic features of study patients are shown in Table 1.

### 3.2. Baseline and control coronary artery diameter

Mean baseline diameters of proximal medial and distal coronary artery segments were similar in the two groups of patients. Baseline coronary diameter before methylergometrine and control diameters before either estradiol 17β or placebo were also similar in the two groups of patients.

### 3.3. Effect of methylergometrine on coronary artery diameter

The intracoronary administration of methylergometrine did not affect systolic and diastolic blood pressure or heart rate in both groups of patients. After administration of methylergometrine, 9 patients showed significant coronary artery constriction (>50% luminal diameter) requiring the infusion of intracoronary nitrates and were therefore excluded from randomisation. The remaining 16 patients showed transient mild (<50%) coronary artery constriction after the highest dose of intracoronary methylergometrine and were randomised to receive either estradiol 17β or placebo.

After first peak methylergometrine infusion (30 μg), coronary artery diameters were reduced by a similar extent in patients allocated to either estradiol 17β or placebo (Fig. 1). Coronary artery diameters were also similar after infusion of estradiol 17β or placebo. No significant changes in coronary artery diameters compared to baseline study were observed at submaximal doses of methylergometrine in patients allocated to estradiol 17β or placebo (Fig. 1). Estradiol 17β but not placebo significantly reduced the degree of coronary artery constriction at peak methylergometrine infusion as compared to baseline study (−0.9±4.5% vs. −19±6%, estradiol 17β vs. placebo $p<0.001$; Fig. 2). Similar results were obtained on coronary artery area at peak methylergometrine infusion. Placebo reduced coronary artery area by 32±10% compared to

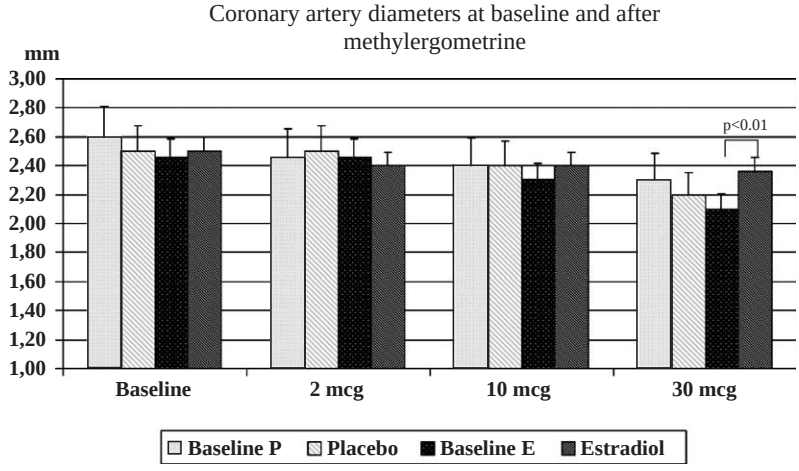


Fig. 1. Proximal coronary artery diameters following intracoronary administration of methylergometrine before and after intracoronary estradiol 17β or placebo. Baseline P=Baseline study in patients randomised to placebo; Baseline E=Baseline study in patients randomised to estradiol 17β; P=Study after placebo infusion; E=Study after estradiol 17β infusion.

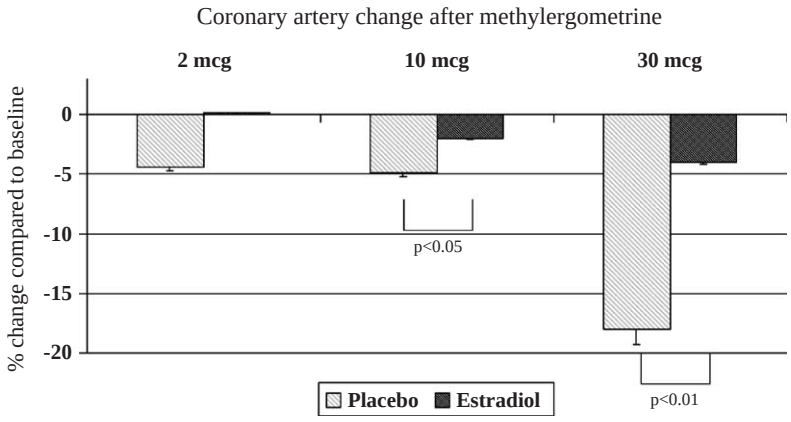


Fig. 2. Percent change of proximal coronary artery diameters following intracoronary methylergometrine infusion after placebo or estradiol 17β. Estradiol 17β significantly attenuated the degree of methylergometrine coronary artery constriction.

baseline study, while after estradiol 17β coronary artery area changed by 3.2±9% ($p<0.001$, as compared to baseline study). No difference in the response of proximal medial and distal segments was observed.

### *3.4. Response to intracoronary nitrates*

Vasodilation of proximal coronary segments after nitrates was 23% in the estradiol group and 19% in the placebo group (NS). Similarly, nitrate-induced dilation of medial and distal segments was not different in the two groups (23% vs. 19%, NS; and 18% vs. 19%, NS). Within groups, dilation of proximal, medial, and distal segments was not significant different.

## 4. Discussion

This study clearly showed an effect of estradiol 17β upon coronary vascular smooth muscle in vivo, in humans. These findings are consistent with the results of previous experimental studies which have shown that estradiol 17β has a relaxing effect upon precontracted rabbit and human coronary artery rings by activation of receptor-operated and potential operated calcium channels [14–17]. The present study shows that acute intracoronary administration of estradiol 17β reduces the degree of methylergometrine-induced coronary artery constriction in menopausal women with coronary artery disease. This effect of estradiol 17β upon coronary reactivity seems to be attributable more to the calcium antagonistic effect of the hormone than to its effect upon the endothelial function. The relieve of methylergometrine-induced vasoconstriction is an immediate effect; thus, supporting the hypothesis of an acute non-genomic modulation of coronary vasomotion.

There is increasing evidence that estrogens are vaso-active substances able to modulate vascular tone of coronary and peripheral arteries. The effect of estrogens in restoring the altered endothelial function has been shown in vitro, in vivo, and in humans in different vascular beds. Volterrani et al. have shown that estradiol 17β reduces peripheral vascular resistances and increases peripheral blood flow in menopausal women [24]. Williams et al. have reported a reversal of the acetylcholine-induced vasoconstriction by estradiol 17β implants in ovariectomised monkeys fed with an atherogenic diet for 30 months [12]. Collins et al. have shown that acute administration of estradiol 17β reverses the coronary constrictor effect of acetylcholine in menopausal women with coronary artery disease and that is gender dependent [21]. Reis et al. demonstrated an increase in coronary blood flow and cross sectional area [19], and decreased resistance in postmenopausal women 15 min after an intravenous infusion of ethinylestradiol. Abnormal coronary vasomotor responses to acetylcholine were attenuated. Similar results were obtained by Gilligan et al. in female patients receiving continuous infusion of estrogens achieving physiological concentrations of intracoronary estradiol 17β [20]. These studies indicate that estrogens influence vascular tone by the production of endothelium-derived relaxing factor (nitric oxide). Estrogen can induce calcium-dependent nitric oxide synthase, increasing its activity causing nitric oxide release [26]. Recently, estrogen receptor has been identified in endothelial cells from human aorta, coronary, and umbilical arteries [27,28]. Using inhibitors of nitric oxide synthase, Tagawa et al. have demonstrated, in humans, that estrogens acutely improve both nitric oxide-mediated and non nitric oxide-mediated vasodilation in the peripheral vasculature of the forearm [29].

Animal and humans in vitro studies have produced evidence that estrogens have calcium antagonistic properties, accounting for a new non-endothelium-dependent mechanism of relaxation of coronary and peripheral arteries. Calcium antagonistic properties of estrogen have been demonstrated in uterine arteries, cardiac myocites, and vascular smooth muscle cells [30,31]. Since it has been proposed that calcium channel blockers may reduce the

progression of atherosclerosis in animals, it has been hypothesised that estrogens might reduce the progression of coronary artery disease by a similar mechanism in humans [32,33]. Jiang et al. have shown that estradiol 17β induces relaxation of isolated coronary artery rings and isolated cardiac myocites contracted both by activation of receptor-operated and potential-operated calcium channels [15–17]. A calcium antagonistic property of estrogen has been confirmed in coronary vascular myocites by measuring cytosolic concentration, contraction, and calcium current [18]. Recently, Sudhir et al. have demonstrated that estrogens cause dilation of conductance and resistance coronary arteries in dogs when administered acutely into the coronary circulation [34]. This in vivo effect was shown to be endothelium-independent and partially mediated by effects on calcium channels.

To our knowledge, the present is the first study to show a non-endothelium-dependent effect of the hormones upon coronary vasculature in vivo in humans. Indeed, methylergometrine causes constriction of coronary arteries via a direct effect upon vascular smooth muscle cells. Therefore, the attenuation of the methylergometrine-induced vasoconstriction by estradiol 17β appears to be dependent upon a direct effect of the hormone on coronary smooth muscle. Because of the short time interval between estradiol 17β administration and the attenuation of methylergometrine-induced vasoconstriction, this effect is unlikely to be genomic. The direct effect of estradiol 17β upon vascular smooth muscle may play an integral role in the acute and chronic regulation of coronary tone, and therefore in the protection of the cardiovascular system. Together with the endothelium-dependent mechanism, the endothelium-independent effect of the hormone may explain the vascular effects of ovarian hormones [35]. In this regard, more human in vivo studies are needed to demonstrate the effective role of this mechanism in vascular diseases related, at least in part, to increased vascular tone.

## References


[1] Stampfer MJ, Colditz GA. Oestrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. Prev Med 1991;20:47–63.

[2] Bush TL, Barrett-Connor E, Cowan LD. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. Circulation 1987;75:1102–9.

[3] Sullivan JM, Vander Zwagg R, Lemp GF. Postmenopausal estrogen use and coronary atherosclerosis. Ann Intern Med 1988;108:358–63.

[4] Wallentin L, Larsson-Cohn U. Metabolic and hormonal effects of postmenopausal oestrogen replacement treatment: II. Plasma lipids. Acta Endocrinol 1977;86:597–607.

[5] Godsland IF, Wynn V, Crook D, Miller NE. Sex, plasma lipoproteins, and atherosclerosis prevailing assumptions and outstanding questions. Am Heart J 1987;114:1467–503.

[6] Tikkanen MJ, Nikkila EA, Kuusi T, Sipinen SU. High-density lipoprotein-2 and hepatic lipase: reciprocal changes produced by oestrogen and norgestrel. J Clin Endocrinol Metab 1982;54:1113–7.

[7] El Seifi S, Green IC, Perrin D. Insulin release and steroid-hormone binding in isolated islets of Langherans in the rat: effects of ovariectomy. J Endocrinol 1981;90:59–67.

[8] Bailey CJ, Ahmed-Sorour H. Role of ovarian hormones in the long-term control of glucose homeostasis. Effects of insulin secretion. Diabetologia 1980;19:475–81.

[9] Gebara OCE, Mittleman MA, Sutherland P. Association between increased oestrogen status and increased fibrinolytic potential in the Framingham Offspring Study. Circulation 1995;91:1952–8.

[10] Fisher GM, Swain ML. Effect of sex hormones on blood pressure and vascular connective tissue in castrated and noncastrated male rats. Am J Physiol 1977;232:H617–21.

[11] Cheng LP, Kuwahara M, Jacobson J, Foegh ML. Inhibition of myointimal hyperplasia and macrophage infiltration by oestradiol in aorta allografts. Transplation 1991;52:967–72.

[12] Williams JK, Adams MR, Klopfenstein HS. Oestrogen modulates responses of atherosclerotic coronary arteries. Circulation 1990;81:1680–7.

[13] Magness RR, Rosenfeld CR. Local and systemic estradiol 17β: Effects on uterine and systemic vasodilation. Am J Physiol 1989;256:E536–42.

[14] Brown AM, Kunze DL, Yatani A. The agonist effect of dihydropyridines on calcium channels. Nature 1984;311:570–2.

[15] Jiang C, Sarrel PM, Lindsay DC, Poole-Wilson PA, Collins P. Endothelium-independent relaxation of rabbit coronary artery by 17β estradiol in vitro. Br J Pharmacol 1991;104:1033–7.

[16] Chester AH, Jiang C, Borland JA, Yacoub MH, Collins P. Estrogen relaxes human epicardial coronary arteries through non-endothelium-dependent mechanism. Cor Art Dis 1995;6:417–22.

[17] Jiang C, Poole-Wilson PA, Sarrel PM, Collins P. Effect of estradiol 17β on contraction, calcium current and intracellular free calcium in guinea-pig isolated cardiac myocytes. Br J Pharmacol 1992;106:739–45.

[18] Han SZ, Karaki H, Ouchi Y, Orimo H. 17β estradiol inhibits calcium influx and calcium release induced by thromboxane A2 in porcine coronary artery. Circulation 1995;91:2619–26.

[19] Reis SE, Gloth ST, Blumenthal RS. Ethynil estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. Circulation 1994;89:52–60.

[20] Gilligan DM, Quyumi AA, Cannon III RO. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. Circulation 1994;89:2545–51.

[21] Collins P, Rosano GMC, Sarrel PM, Ulrich L, Adamopoulos S, Beale CM, et al. Estradiol 17β attenuates acetylcholine-induced coronary arterial constriction in women but not in men with coronary heart disease. Circulation 1995;92:24–30.

[22] Rosano GMC, Sarrel PM, Poole-Wilson PA, Collins P. Beneficial effect of oestrogen on exercise-induced myocardial ischemia in women with coronary artery disease. Lancet 1993;342:133–6.

[23] Killam AP, Rosenfeld CR, Battaglia FC, Makowski EL, Meschia G. Effect of oestrogens on the uterine blood flow of oophorectomized ewes. Am J Obstet Gynecol 1973;115:1045–52.

[24] Volterrani M, Rosano GMC, Coats A, Beale C, Collins P. Estrogen acutely increases peripheral blood flow in postmenopausal women. Am J Med 1995;99:119–22.

[25] Ryan TJ, Faxon DP, Gunnar RM, Kennedy JW, King III SB, Loop FD, et al. Guidelines for percutaneous transluminal coronary angioplasty. A report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures (Subcommittee on Percutaneous Transluminal Coronary Angioplasty). Circulation 1988 (Aug);78(2):486–502.

[26] Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S. Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc Natl Acad Sci U S A 1994;91:5212–6.

[27] Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism

for steroid hormone regulation of endothelial function. Circulation 1996;94:727–33.
[28] Kim-Schulze S, McGowan KA, Hubchack SC, Cid MC, Martin MB, Kleinman HK, et al. Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. Circulation 1996; 94:1402–7.
[29] Tagawa H, Tagawa T, Shimokawa H. Estrogen acutely improves both nitric oxide-mediated and non nitric oxide-mediated endothelium-dependent vasodilation in postmenopausal women. Circulation 1995; 92(8):I-68.
[30] Stice SI, Ford SP, Rosazza JP, Van Orden DE. Role of 4-hydroxylated oestradiol in reducing $Ca^{2+}$ uptake of uterine arterial smooth muscle cells through potential-sensitive channels. Biol Reprod 1987;36:361–8.
[31] Stice SI, Ford Sp, Rosazza JP, Van Orden DE. Interaction of 4-hydroxylated oestradiol and potential-sensitive $Ca^{2+}$ channels in altering uterine blood flow during the oestrous cycle and early pregnancy in gilts. Biol Reprod 1987;36:369–75.
[32] Henry PD, Bentley KI. Suppression of atherogenesis in cholesterol fed rabbits treated with nifedipine. J Clin Invest 1981;68:1366–9.
[33] Lichtlen PR, Hughenoltz PG, Hecker H, Jost S, Deckers JW. Retardation of angiographic progression of coronary artery disease by nifedipine. Lancet 1990;335:1109–13.
[34] Sudhir K, Chou TM, Mullen WL, Hausmann D, Collins P, Yock PG, et al. Mechanism of estrogen-induced vasodilation: in vivo studies in canine coronary conductance and resistance arteries. J Am Coll Cardiol 1995;26(3):807–14.
[35] Kawano H, Motoyama T, Hirai N, Kugiyama K, Ogawa H, Yasue H. Estradiol supplementation suppresses hyperventilation-induced attacks in postmenopausal women with variant angina. J Am Coll Cardiol 2001;37:735–40.