JACC Vol. 29, No. 4
March 15, 1997:800–7

EXPERIMENTAL STUDIES

Testosterone Worsens Endothelial Dysfunction Associated With Hypercholesterolemia and Environmental Tobacco Smoke Exposure in Male Rabbit Aorta

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Objectives. **To assess the effects of interaction of sex hormones, hypercholesterolemia (HC) and environmental tobacco smoke (ETS) exposure on endothelium-dependent relaxation, we examined vascular reactivity in vitro in an animal model of atherogenesis.**

Background. **Animal and human studies indicate the presence of interactions between classic coronary artery disease risk factors and endothelium-dependent relaxation. Sex hormones have also been shown to influence release of endothelium-derived relaxing factor.**

Methods. **New Zealand White rabbits were randomized to receive either an HC diet (n = 8) or ETS exposure plus HC diet (n = 8). Eight rabbits receiving a normal diet, without exposure to ETS, served as the control group. The HC diet consisted of 3% soybean oil and 0.3% cholesterol by weight over 13 weeks. The source of ETS was sidestream smoke of 4 cigarettes/15 min, 6 h/day, 5 days/week over 10 weeks in a smoking chamber. Rabbits were killed, and fresh aortic rings were harvested and maintained in oxygenated Krebs solution in an organ bath at 37°C. Rings were precontracted with norepinephrine and exposed to acetylcholine in increasing doses, and isometric tension was recorded. Rings were also exposed to physiologic concentrations (1 nmol/liter) of either 17-beta-estradiol, testosterone or progesterone before precontraction with norepinephrine and relaxation with acetylcholine. Endothelium-independent relaxation was studied using nitroglycerin. The surface area of the ring covered by lipids was measured by Sudan IV staining.**

Results. **HC and ETS significantly reduced endothelium-dependent relaxation ($p = 0.01$ and $p < 0.0005$, respectively) and caused atherogenesis ($p < 0.0005$ and $p = 0.047$, respectively) but did not affect endothelium-independent relaxation. Incubation with estradiol and estradiol plus progesterone did not influence endothelium-dependent relaxation. Testosterone reduced endothelium-dependent relaxation ($p = 0.049$) and augmented the endothelial dysfunction associated with ETS exposure and HC ($p = 0.03$).**

Conclusions. **Both HC and ETS are atherogenic and impair endothelial function but do not affect endothelium-independent relaxation. Physiologic levels of estradiol and estradiol plus progesterone do not affect endothelium-dependent relaxation. Physiologic levels of testosterone impair relaxation and augment the endothelial dysfunction associated with ETS exposure and HC.**

(J Am Coll Cardiol 1997;29:800–7)

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Impaired endothelial function is thought to be an early event in the development of atherosclerosis. Endothelium-derived relaxing factor (EDRF)–mediated vasorelaxation is impaired by hypercholesterolemia (HC) in both experimental animal models (1,2) and in humans (3–5) and by tobacco smoking (3,6–8). More recently, passive exposure to environmental tobacco smoke (ETS) has been shown in animal models (9) and in humans (10) to be associated with impaired EDRF-mediated vascular relaxation. Furthermore, ETS has also been shown (11,12) to promote atherogenesis in the HC rabbit model.

Sex hormones alter vascular behavior and may mediate gender differences in cardiovascular disease. Estrogens have been shown to be acute vasodilators of the peripheral (13,14), cerebral (15,16) and coronary vasculature (17–25). In vitro studies demonstrate that physiologic levels of estrogens acutely increase release of EDRFs, in particular nitric oxide (NO). Basal release of nitric oxide from aortic rings is greater in female than in male rabbits (26). In vivo studies of atherosclerotic coronary arteries in primates (20–22) and humans (23)

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All editorial decisions for this article, including selection of referees, were made by a Guest Editor. This policy applies to all articles with authors from the University of California San Francisco.

Manuscript received May 29, 1996; revised manuscript received November 20, 1996, accepted December 4, 1996.

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Published by Elsevier Science Inc.

0735-1097/97/$17.00
PII S0735-1097(96)00570-0

Abbreviations and Acronyms

ANOVA = analysis of variance
CAD = coronary artery disease
EC50 = concentration at half-maximal response
EDRF = endothelium-derived relaxing factor
ED80 = dose (concentration) to achieve 80% response
ETS = environmental tobacco smoke
GLM = general linear model
HC = hypercholesterolemia, hypercholesterolemic
LDL = low density lipoprotein
NO = nitric oxide

have demonstrated that estrogens acutely augment vasorelaxation induced by agents such as acetylcholine and serotonin, which induce EDRF release. Studies of guinea pigs (27) suggest that both endothelial and neuronal NO synthase are subject to regulation by estrogen (27). Preliminary studies (28) in perimenopausal women have suggested that estrogen supplementation increases basal NO release in the peripheral vasculature.

Active (29–31) and passive (32) tobacco smoking may be a greater risk factor for cardiac disease in men than women. However, the impact of gender on the effects of passive exposure to ETS is unclear. Furthermore, the interaction between physiologic concentrations of sex hormones and pathophysiologic vascular states such as HC is not well defined. We hypothesized that impairment of EDRF release by HC and ETS would be differentially modulated by male and female sex hormones. Therefore, in the present study we sought to establish the effects of different sex hormones on endothelium-mediated relaxation in normal, HC and ETS-exposed/HC rabbits.

Methods

Protocol. The protocol was approved by the Institutional Committee on Animal Research. Three groups of male New Zealand White rabbits were studied: normal rabbits (n = 8), HC rabbits (n = 7) and HC rabbits exposed to ETS (n = 8).

HC and ETS-exposed/HC rabbits were rendered HC by a high cholesterol diet over 13 consecutive weeks. The cholesterol diet (Ziegler Bros., Inc.) consisted of 3% soybean oil and 0.3% cholesterol by weight. After 3 weeks of the high cholesterol diet, induction of HC was confirmed by serum testing. At 3 weeks, rabbits were randomized to continue receiving the high cholesterol diet (13 weeks) alone or a high cholesterol diet (13 weeks) plus ETS exposure (weeks 3 to 13). Blood samples were taken through ear venipuncture for serum cholesterol at the start of the study and at 3, 9 and 13 weeks (the last measure at euthanasia).

Rabbits were housed in individual cages, and those randomized to the ETS-exposed/HC group were placed in ETS exposure chambers (model H 5500, BioClean, Lab Products Inc.), 1.92 m × 1.92 m × 0.97 m (3.58 m^3) that accommodated eight rabbits. Rabbits were exposed to sidestream smoke from Marlboro filter cigarettes (4 cigarettes/15 min for 6 h/day, 5 days/week) using a smoking machine (Heinr, Borgwald GMBH RM 1/G, Hamburg, Germany) for 10 weeks from weeks 3 to 13. Three fans in the exposure chambers were adjusted to ensure good air mixing within the smoking chamber. At the end of the 6-h exposure period, the exhaust fan on the BioClean unit was turned on and rapidly lowered the level of ETS pollution in the exposure chamber to background levels corresponding to those of the control animals until the next day, when the BioClean unit was turned off and the smoking machine was turned on again. Rabbits randomized to the HC group were placed in separate cages in the same type of exposure chamber in another room without a smoking machine.

Eight normal male New Zealand White rabbits, age matched, fed a regular rabbit chow diet, served as normal control animals.

Monitoring smoke exposure inside chambers. We monitored the constituents of ETS (carbon monoxide, total particulate matter, respirable suspended particulates and air nicotine), as previously described (33).

Hematologic and biochemical analysis. Total serum cholesterol, low density lipoprotein (LDL) cholesterol and triglyceride levels were determined by automated enzymatic methods (Coulter DART cholesterol reagent using the Dacos and Dacos XL analyzers). The blood samples were drawn in the morning (Tuesday to Friday) after 12 h of fasting and before ETS exposure.

Harvesting of aortic rings. At week 21, rabbits were killed by lethal injection with intravenous administration of pentobarbital (130 mg/kg body weight) in accordance with our institutional guidelines. The aorta was rapidly removed from its origin (2 cm distal to the aortic valve) down to the bifurcation of the internal iliac arteries. The segment immediately distal to the left subclavian artery was used in all animals for measuring vascular reactivity. The aorta was dissected free of connective tissue and fat. Arterial rings (2- to 3-mm diameter) were cut into 5- to 7-mm lengths. One ring per animal was studied. Each ring was suspended horizontally between two stainless steel parallel wires for the measurement of isometric tension, in individual organ baths containing Krebs solution, composed of (mmol/liter): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, K_2PO_4 1.2 and glucose 11.1, bubbled with 95% O_2 and 5% CO_2. Bath temperature was maintained at constant 37°C. Two side by side quadruple Radnotti organ bath systems (Radnotti Glass Technologies Inc.) were used. The isometric tension generated by the ring segment was measured using Radnotti high sensitivity isometric transducers (TRN001) and recorded continuously on an eight-channel MacLab/8e recording system (Analog Digital Instruments, Inc.) on MacLab Chart v3.3 (Analog Digital Instruments, Inc.) recording software.

Vascular responsiveness to norepinephrine, acetylcholine and nitroglycerin. Ring segments were stabilized at 4 g of rest tension for 60 min before the study began. To measure

responsiveness to norepinephrine and to calculate the dose needed for precontraction, norepinephrine in increasing doses (from 10^{-9} to 10^{-4} mol/liter) was added to each ring per bath. For each ring, the dose of norepinephrine needed to achieve 80% maximal contraction (ED80) was calculated. The baths were rinsed three times with fresh Krebs solution, and the rings were allowed to stabilize for 1 h.

To determine endothelium-derived nitric oxide–mediated vasorelaxation, aortic rings were exposed to acetylcholine. Acetylcholine in increasing doses (from 10^{-9} to $10^{-4.5}$ mol/liter) was added to the organ baths after the rings had been precontracted by the ED80 dose of norepinephrine, and stable tension had developed. At the end of the acetylcholine series, the baths were rinsed twice with fresh Krebs solution, and the rings were allowed to stabilize at baseline tension.

To determine endothelium-independent relaxation, nitroglycerin in increasing doses (from 10^{-9} to 10^{-5} mol/liter) was added to the organ baths, after the rings had been precontracted by the ED80 dose of norepinephrine, and stable tension had developed. At the end of the nitroglycerin series, the organ baths were rinsed twice with fresh Krebs solution, and the rings were allowed to stabilize at baseline tension.

Effects of sex hormone incubation on acetylcholine-induced relaxation. The effect of sex steroids on endothelium-mediated relaxation was tested by incubation with physiologic concentrations (1 nmol/liter) of either 17-beta-estradiol, testosterone or 17-beta-estradiol plus progesterone 30 min before precontracting the ring with the EC80 dose of norepinephrine, followed by the addition of acetylcholine (doses 10^{-9} to $10^{-4.5}$ mol/liter). Estradiol is known to acutely improve acetylcholine-induced endothelium-dependent relaxation of the female arterial vasculature within 20 to 30 min (21,23).

At the end of the organ bath experiment, the used ring segment was preserved in 10% formalin solution for 24 h before Sudan IV staining for determination of intimal lesion area. The percentage of the surface involved by intimal lesions was measured by planimetry and expressed as the percentage of the overall area.

Drugs. Norepinephrine, acetylcholine, 17-beta-estradiol, testosterone and progesterone were purchased from Sigma Chemical Company. Nitroglycerin was purchased from Solopak Laboratories Inc. Distilled water was used as the solvent for norepinephrine, acetylcholine and nitroglycerin. Dimethlsulfoxide was used to initially dissolve 17-beta-estradiol, testosterone and progesterone. Distilled water was used to perform subsequent dilutions of the sex steroids.

Histologic Measurement. At the end of the organ bath study, the ring was opened, pinned with the endothelial side facing a cork board and placed in a normal saline bath. The ring segment was fixed in 10% formalin and stained with the lipophilic stain Sudan IV and photographed. The stained lesions were estimated quantitatively by planimetry of the sudanophilia in the photographs. The planimetric measurements were made without knowledge of other data and were calculated as the mean of two determinations.

Calculations and statistical analysis. Rabbits were killed and aortic rings harvested in random order, and the ring/organ bath experiments and data recording were performed by an observer (S.J.H.) blinded to treatment group (normal, HC, ETS-exposed/HC rabbits). Relaxation responses were expressed as percent change of net developed tension, concentration at half-maximal response (EC50) and slope. Response to norepinephrine was expressed as change in tension (g). A curve of best fit was calculated for each ring, using the equation for the Hill coefficient, and EC50 and slope were derived (Kaleidagraph, version 3.0, Synergy Software).

One ring from the testosterone-incubated ETS-exposed/HC group was not included in the analyses because its maximal response to acetylcholine was >3 SD from the mean value for that group.

The effects of HC and ETS on animal weights, intimal lesions area and vascular reactivity were evaluated using a general linear model (GLM) analysis of variance (ANOVA) (Minitab Version 10.2, GLM procedure), including the presence or absence of cholesterol and ETS as main effects.

The effects of sex hormone incubation (no hormone, 17-beta-estradiol alone, 17-beta-estradiol/progesterone combination, testosterone) on maximal acetylcholine-induced relaxation were evaluated using GLM ANOVA, including (the presence or absence of) 17-beta-estradiol, progesterone and testosterone as main effects, the ETS–sex hormone interaction and the HC–sex hormone interaction. Testing the significance of the interaction term specifically permitted us to test whether the effects of ETS exposure were modified by the presence of sex hormone incubation (beyond purely additive effects).

Because an ETS–testosterone effect on maximal relaxation induced by acetylcholine was seen on the GLM ANOVA, indicating that testosterone altered the effect of ETS on relaxation, we tested for differences in the ETS-exposed/HC group using one-way ANOVA with post hoc Student-Newman-Keuls testing.

Because we previously published data on cholesterol values in normal rabbits (33), we did not collect these data in the present study. Therefore, only cholesterol values for HC and ETS-exposed/HC rabbits were compared with a *t* test. A p value <0.05 was considered significant. Results are expressed as mean value ± SE.

Results

Animal data (Table 1). *Body weight and food intake.* Ingestion of a high cholesterol diet caused greater body weight ($p = 0.001$), most likely because of the higher caloric value of the cholesterol-rich diet. ETS did not affect body weight ($p = 0.67$) but did reduce food intake ($p = 0.01$).

Cholesterol levels. Total cholesterol levels of the ETS-exposed/HC group were higher than those of the HC group (1,359 ± 203 vs. 820 ± 133 mg/dl, $p = 0.04$) but LDL cholesterol (111 ± 27 vs. 89 ± 19 mg/dl, respectively, $p = 0.53$)

Table 1. Body Weight, Food Ingestion, Serum Lipoprotein Levels and Extent of Intimal Lesions in the Three Study Groups

	Normal Group (mean ± SE)	HC Group (mean ± SE)	ETS-Exposed/HC Group (mean ± SE)	p Value
Weight (kg)	3.56 ± 0.05	4.04 ± 0.10	3.99 ± 0.11	0.001* 0.67†
Cholesterol (mg/dl)		710 ± 196	1,359 ± 182	0.04
TGs (mg/dl)		66 ± 7	89 ± 11	0.53
LDL (mg/dl)		89 ± 19	111 ± 27	0.09
Food (g/day)		1.67 ± 11	1.30 ± 8	0.01
Air CO (ppm)	0.2 ± 0.1	0.2 ± 0.1	88.7 ± 2.6	< 0.0005† 0.99*
Particles (mg/m^3)	1.0 ± 0.0	1.0 ± 0.0	53.5 ± 1.5	< 0.0005† 0.99*
Intimal lesions (% surface area)	0	35.7 ± 12.6	49.1 ± 7.6	< 0.0005* 0.047†

*Normal versus hypercholesterolemic (HC) group. †Hypercholesterolemic versus environmental tobacco smoke (ETS)–exposed/hypercholesterolemic group. LDL = low density lipoprotein cholesterol; TGs = triglycerides.

and triglyceride levels (89 ± 11 vs. 66 ± 7 mg/dl, respectively, $p = 0.09$) of both groups were similar.

ETS exposure. ETS exposure resulted in greater particulate and carbon monoxide exposure.

Extent of intimal lesions. HC induced atherogenesis in the aorta ($p < 0.0005$), and ETS induced further atherogenesis ($p = 0.047$) (Fig. 1).

Vascular reactivity (Table 2). *Effect of smoking and HC on vascular relaxation.* HC impaired endothelium-dependent relaxation ($p = 0.01$) but did not affect endothelium-independent relaxation ($p = 0.20$). ETS further impaired endothelium-dependent relaxation ($p < 0.0005$) but did not affect endothelium-independent relaxation ($p = 0.66$) (Fig. 1).

Effect of incubation physiologic concentrations of sex steroids on vascular relaxation. Incubation with testosterone alone reduced acetycholine-induced endothelium-dependent relaxation ($p = 0.049$), did not affect the HC-induced impairment of endothelium-dependent relaxation (HC–testosterone interaction: $p = 0.65$) but accentuated the ETS-induced impairment of endothelium-dependent relaxation (ETS–testosterone interaction: $p = 0.03$). Maximal relaxation in response to acetylcholine with testosterone incubation in the ETS-exposed/HC group was significantly less ($p < 0.05$) than that in response to acetycholine alone or acetylcholine with other hormone incubation in the ETS-exposed/HC group.

Incubation with estrogen alone did not affect endothelium-dependent relaxation ($p = 0.50$) or ETS- or HC-induced impairment of endothelium-dependent relaxation (HC–

Figure 1. Effect of ETS and HC on intimal lesions and endothelium-dependent and endothelium-independent relaxation. **Left,** Atherosclerosis: effect of increasing the percent of surface covered by lipid. **Middle,** Endothelium-dependent relaxation: effect of decreasing acetylcholine-induced relaxation of aortic rings. **Right,** Endothelium-independent relaxation: No change in nitroglycerin-induced relaxation is seen.

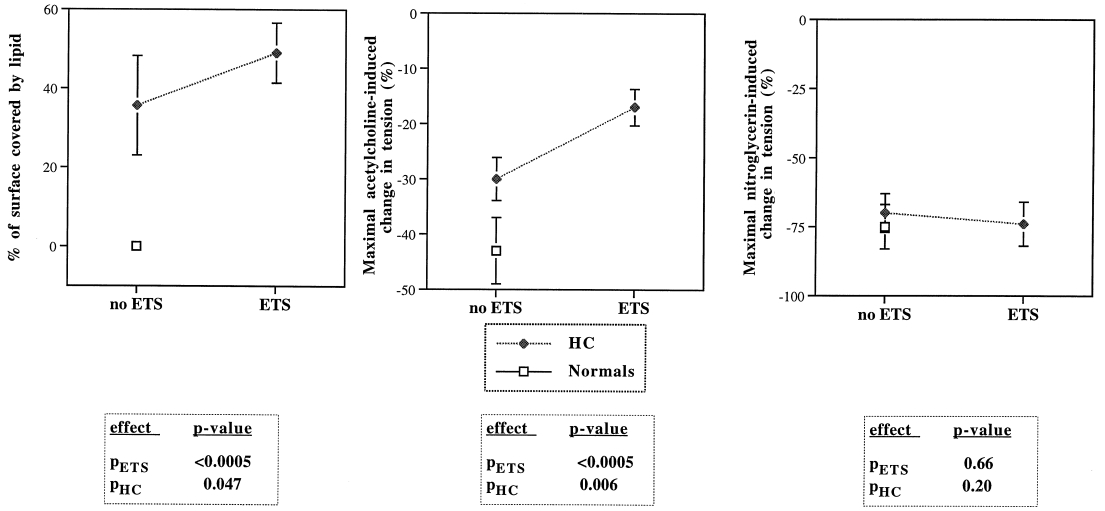


Table 2. Maximal Relaxation Responses to Acetylcholine in the Presence of Various Sex Hormones

	Normal Group (n = 8) (mean ± SE)	HC Group (n = 7) (mean ± SE)	ETS-Exposed/ HC Group (n = 8) (mean ± SE)
ACh	−43 ± 6.8	−30 ± 3.9	−17 ± 3.3*
ACh+E2	−40 ± 5.1	−30 ± 5.9	−16 ± 3.6*
ACh+E2/Prog	−35 ± 5.4	−28 ± 6.0	−12 ± 2.1*
ACh+Testo	−38 ± 5	−32 ± 6.1	−3.5 ± 1.6†
Nitroglycerin	−75 ± 8	−70 ± 7	−74 ± 8

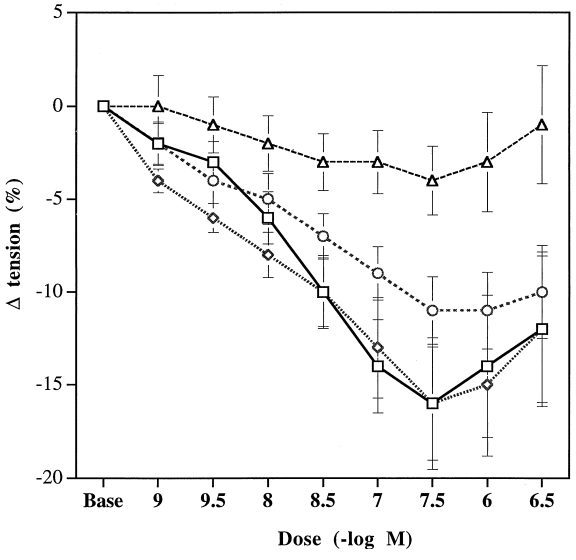
ACh = acetylcholine; E2 = 17-beta-estradiol; E2/Prog = 17-beta-estradiol and progesterone; ETS = environmental tobacco smoke; HC = hypercholesterolemia. $p_{HC} = 0.006$; $p_{ETS} < 0.0005$; $p_{E2} = 0.50$; $p_{E2/Prog} = 0.89$; $p_{Testo} = 0.049$. *Less than †.

estradiol interaction: p = 0.70; ETS–estradiol interaction: p = 0.22). Incubation with estrogen plus progesterone did not affect endothelium-dependent relaxation (p = 0.89) or the ETS- or HC-induced impairment of endothelium-dependent relaxation (HC–estradiol plus progesterone interaction: p = 0.81; ETS–estradiol plus progesterone interaction: p = 0.43) (Fig. 2, 3).

Discussion

The present study demonstrates that in an experimental model of atherogenesis, 1) both HC and ETS induce atherogenesis and endothelial dysfunction but do not alter endothelium-independent relaxation. 2) Incubation with physiologic levels of 17-beta-estradiol and 17-beta-estradiol/progesterone does not influence endothelium-dependent relaxation nor does it alter the endothelial dysfunction induced by HC or ETS exposure. 3) Incubation with physiologic levels of testosterone reduces endothelium-dependent relaxation and augments the endothelial dysfunction induced by ETS. Incubation with physiologic levels of testosterone does not alter the endothelial dysfunction induced by HC.

Figure 2. HC rabbits with ETS exposure: dose–response curves of acetylcholine-induced relaxation (net change [Δ] in developed tension) alone (**squares**) and with 17-beta-estradiol (**diamonds**), testosterone (**triangles**) or 17-beta-estradiol and progesterone incubation (**circles**). Incubation with testosterone resulted in marked attenuation of acetylcholine-induced vasorelaxation.



The goal of the present study was to examine the effect of physiologic levels of sex hormones on vascular relaxation in an animal model of HC and ETS exposure. We confirmed previously published studies (34–36) that demonstrated that HC induces endothelial dysfunction and atherogenesis and studies (11,12,37) that demonstrate that ETS exposure induces endothelial dysfunction and atherogenesis.

In the present study, we examined the vascular effects of physiologic effects of sex hormones. In male rabbits, estrogens did not improve the impaired acetylcholine-mediated relaxation in the ETS-exposed/HC group. This finding may indicate that the beneficial effect of estrogens does not extend beyond the estrogen-deficient postmenopausal setting or that it is not seen in men.

Testosterone has complex effects on vascular reactivity and in some models has been associated with enhanced vasoconstrictor action (38,39). Data on the effects of testosterone on atherosclerosis and vascular reactivity are conflicting. In female postmenopausal lipid-fed cynomolgus monkeys, Adams et al. (24) showed that testosterone supplementation was associated with significantly greater atherosclerosis, although it was also associated with improvement in vascular reactivity. Shror et al. (38) demonstrated in guinea pigs in vitro that testosterone can enhance coronary artery vascular reactivity to thromboxane A_2. Matsuda et al. (40) and Masuda et al. (41) showed that testosterone increases thromboxane A_2 receptor density and responsiveness in rat aorta and platelets. In an older study, Greenberg et al. (39) demonstrated increased responses to norepinephrine and tyramine after testosterone injection in the canine vasculature.

Studies in animal models of atherosclerosis have shown that progesterone supplementation has variable effects on acetylcholine-induced relaxation. Williams et al. (21) demonstrated in postmenopausal lipid-fed female cynomolgus monkeys that addition of cyclic or continuous medroxyprogesterone acetate to conjugated equine estrogen regimens diminished endothelium-mediated dilation in response to acetylcholine. Miller and Vanhoutte (42) showed that progesterone itself had little effect on vasorelaxation but that it antagonized estrogen-induced vasorelaxation. Our study does not support the observation that the addition of progesterone to estradiol has a deleterious effect on endothelium-dependent relaxation.

Clinical applications. Tobacco smoking (43) and HC (44,45) are well established risk factors for coronary artery disease (CAD). An association between increase in CAD events and mortality and exposure to ETS has been reported (32,46–55), suggesting that the coronary mortality rate in U.S. never-smokers is increased 20% to 70% by passively breathing

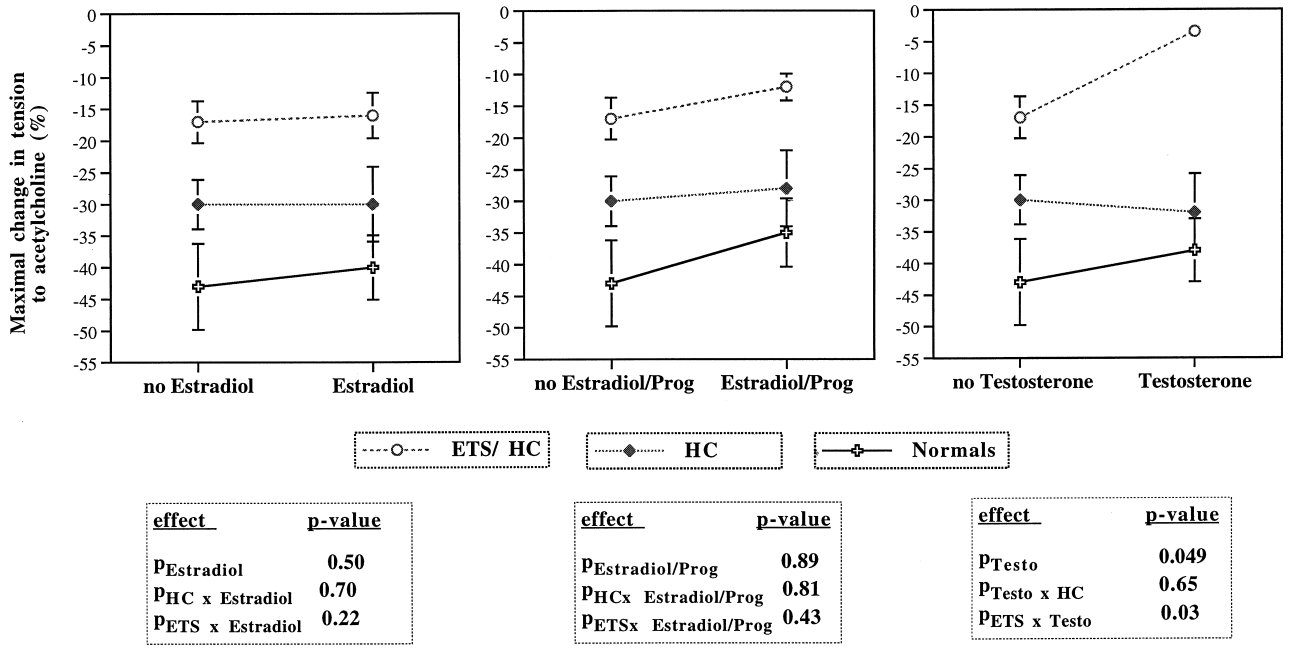


Figure 3. Effect of hormone incubation on endothelium-dependent relaxation of normal, HC and ETS-exposed/HC rabbit aorta. An HC and ETS effect of reducing endothelium-dependent relaxation is seen. **Left,** No effect of estradiol incubation on endothelium-dependent relaxation is seen. **Middle,** No significant effect of estradiol/progesterone (Prog) incubation on endothelium-dependent relaxation is seen. **Right,** Testosterone (Testo) incubation of ETS-exposed/HC rabbit aorta reduces relaxation but has no effect on HC and normal rabbit aorta.

tobacco smoke (56) and that passive ETS exposure constitutes a risk for CAD.

Sex hormones may influence the incidence of atherosclerotic vascular disease. There is a significant gender difference in the incidence of CAD that is believed to be mediated in part by sex hormones. It is generally thought that the presence of estrogens imparts clinically important benefits on the course of CAD (57).

However, it may be that testosterone in some situations imparts a deleterious effect on vascular disease, such as in the presence of smoking. The number of smoking-attributable deaths in each state of the United States is twice as high for male as for female smokers (29). The role of cigarette smoking in thromboangiitis obliterans (Beurger's disease), predominantly a disease of male smokers, is well established (58), and more smoking-related deaths occur in male than in female smokers (30). This gender-related difference in mortality may be explained by the greater number of male smokers and the fact that they smoke more cigarettes than do female smokers (30). However, some data suggest that males smokers have a greater increase in risk. In the American Cancer Society 25-state study (Current Population Study [CPS-1]), the estimated relative risk of coronary heart disease mortality for current male smokers >35 years old was 1.83 (95% confidence interval [CI] 1.76 to 1.91) and for those 35 to 64 years old, 2.25 (95% CI 2.13 to 2.39) (30). The estimated relative risk for female smokers was 1.40 (95% CI 1.29 to 1.51) and 1.81 (95% CI 1.67 to 1.97), respectively. In the CPS-II trial, the estimated relative risk of coronary heart disease mortality for current male smokers >35 years old was 1.94 (95% CI 1.80 to 2.08), and that for current female smokers was 1.78 (95% CI 1.62 to 1.97). Steenland et al. (32) recently published the CPS-II data on passive smoking, which also suggest that men exposed to ETS tended to have greater risk of vascular events than women. Thus, male smokers may have greater susceptibility to the complications of vascular disease (30), and the results of the present study suggest that this susceptibility may be mediated by a testosterone effect, particularly by a testosterone–ETS interaction.

Limitations of the study. In the present study, only male rabbits were studied. We do not know whether the interactions between sex hormones, HC and ETS in aortic rings from female rabbits would be different.

Acetylcholine induces relaxation by stimulation of endothelial muscarinic receptors and subsequent release of nitric oxide. However, endothelium-dependent vasorelaxation may be mediated through other mechanisms, such as the arachadonic acid/prostacyclin pathway. We did not explore the contribution of these other potential mechanisms to the impairment of endothelial function by HC or ETS, or both, or their potential modulation by sex hormones.

Conclusions. ETS exposure and HC cause atherogenesis and endothelial dysfunction. Short-term exposure to physiologic concentrations of testosterone worsens endothelial dysfunction and prominently augments the endothelial dysfunction induced by ETS exposure. Further studies are needed to elucidate the clinical interaction between ETS, HC and sex hormones in populations predisposed to vascular disease.

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