Response to sex hormones differs in atherosclerosis-susceptible and -resistant mice

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Potier, Mylène, Michael Karl, Sharon J. Elliot, Gary E. Striker, and Liliane J. Striker. Response to sex hormones differs in atherosclerosis-susceptible and -resistant mice. Am J Physiol Endocrinol Metab 285: E1237–E1245, 2003. First published August 12, 2003; 10.1152/ajpendo.00451.2002.—Genetic factors that determine the degree of susceptibility to atherosclerosis may also influence the effects of estrogens and progestins in arterial vessel disease. We examined and compared estrogen receptor (ER) and progesterone receptor (PR) expression and the effects of 17β-estradiol (E2) and progesterone (P) on collagen synthesis and matrix metalloproteinase (MMP) activities in the aortic arch and in cultured aortic smooth muscle cells (ASMC) of atherosclerosis-susceptible (C57Bl6/J, B6) or -resistant (C3H/HeJ, C3H) mice. ERα, ERβ, and PR levels were higher in the aorta and ASMC of atherosclerosis-susceptible B6 mice. In transfection studies using an estrogen response element-driven reporter plasmid, E2 elicited a 2-fold increase in luciferase activity in ASMC of B6 (B6-ASMC), which demonstrated the transcriptional activity of ER in atherosclerosis-susceptible cells. Importantly, the response of endogenous target genes to E2 and P was different in B6-ASMC and C3H-ASMC. E2 decreased collagen synthesis but had no effect on MMP activities in B6-ASMC. P decreased MMP-2 and MMP-9 activity in B6-ASMC. In contrast, E2 increased MMP-2 and decreased MMP-9 activity but had no effect on collagen synthesis in C3H-ASMC. P had no effect on collagen synthesis and MMP activity in C3H-ASMC. These differences in response to sex hormones may have important implications for women who receive hormone replacement therapy.

C3H/HeJ (C3H) mice are resistant to diet-induced atherosclerosis. Comparative analysis of these mouse models provides a unique opportunity to study the impact of the genetic background on the expression of female sex hormone receptors and their effects on molecules involved in the protection or progression of atherosclerosis such as collagens and matrix metalloproteinase (MMP). An imbalance of collagen synthesis and degradation by MMP in the vascular wall have an important role in the pathogenesis of atherosclerosis (4). An altered response to estrogens and/or progestins may contribute to this imbalance, since these sex steroids are known to regulate collagen synthesis and MMP activity in reproductive organs (19, 29) and in large and small vascular beds (30), including the renal glomerulus (7, 17, 26).

The development of cultured aortic smooth muscle cells (ASMC) now provides the means to dissect their contribution to the susceptibility and resistance to atherosclerosis, in part by studying the molecular mechanisms involved in the regulation of collagen synthesis and MMP activity by sex hormones.

Hormone replacement therapy (HRT), consisting of estrogens and progestins, had been advocated for the primary and secondary prevention of atherosclerosis in postmenopausal women on the basis of observational data. Unexpectedly, the Heart and Estrogen/progestin Replacement Study (HERS and HERS II) and the Estrogen Replacement in Atherosclerosis trial (ERA) showed that HRT was not beneficial in women with established CHD (6, 9, 10). More recently, the estrogen and progestrone treatment arm of the Women’s Health Initiative (WHI) was interrupted because the risks outweighed the potential net benefits for primary prevention in women without clinically known CHD (31).

Thus there exists an obvious discrepancy between the negative results of the randomized trials and the benefits of HRT previously reported in observational studies. The women who were studied in the HERS and ERA trials had preexisting CHD; i.e., this patient population had a demonstrated susceptibility to developing atherosclerosis. These women did not respond favorably to HRT. Furthermore, in the WHI study,
there was no overall benefit and an increased relative risk in cardiac events in women without prior clinical evidence of CHD who received HRT for primary prevention. The differences between the outcomes of the randomized and observational studies might be partly due to the fact that women differ in terms of their underlying susceptibility to atherosclerosis.

Therefore, we hypothesized that the genetic background may account for the different outcomes of HRT in individuals susceptible or resistant to atherosclerosis. We investigated whether estrogens and progestins differently regulated genes involved in the pathogenesis of atherosclerosis, such as collagen and MMP. We examined and compared whole aortic arch tissue and ASMC isolated from the aortic arch of young female atherosclerosis-resistant C3H and atherosclerosis-susceptible B6 mice. Surprisingly, estrogen receptor α and β (ERα and ERβ) and progesterone receptor (PR) expression was higher in the aortic arch tissue and ASMC isolated from atherosclerosis-susceptible B6 mice. The higher ER levels were paralleled by a higher transcriptional response to 17β-estradiol (E2) when ASMC isolated from atherosclerosis-susceptible B6 mice were transfected with a synthetic estrogen response element (ERE) containing luciferase-based reporter gene. Importantly, E2 and progesterone (P) differentially regulated type IV and type I collagen synthesis and MMP-2 and MMP-9 expression and activity in ASMC isolated from atherosclerosis-resistant and -susceptible mice. Therefore, the complexity of genetically determined differences in response to estrogens, as illustrated by our results in the murine model, could account for the contradictory and often negative results obtained with HRT in postmenopausal women.

**EXPERIMENTAL PROCEDURES**

**Isolation of aorta.** Eight-week-old female B6 (n = 3) and C3H (n = 3) mice were ordered from Jackson Laboratory (Bar Harbor, ME). At death, the mean weights (17.0 ± 0.4 and 19.0 ± 1.5 g, respectively, for B6 and C3H) and blood pressures (data not shown) between the groups did not differ significantly. The aortic arches were collected, cut into fragments, and kept at −80°C until they were processed for mRNA analyses. A fragment of aortic arch was immediately placed at 37°C onto a fibronectin (Collaborative Biomedical, Bedford, MA)-coated well (191 mm²) in DMEM-F12 medium containing 20% FBS (Life Technologies, Grand Island, NY) for ASMC isolation.

**Cell culture.** ASMC were isolated from the aortic arch of C3H (C3H-ASMC) and B6 (B6-ASMC) female mice (8 wk old). After propagation for three passages, the cells were characterized by positive staining with anti-smooth muscle α-actin (Sigma, St. Louis, MO). ASMC were subcultured in DMEM-F12 supplemented with 20% FBS (Life technologies) and used between passages 6 and 16. A second set of cultured ASMC independently isolated from a different mouse was used to confirm mRNA and protein expression. In all experiments designed to examine E2 (Sigma) and P (Sigma) effects, ASMC were transferred into phenol red-free medium (Life Technologies) supplemented with charcoal-stripped FBS (Hyclone, Pittsburgh, PA). Proliferation was assessed in the presence of E2 or P (0, 0.1, 1, and 10 nmol/l). Cell number determined at days 1 and 3 with a Coulter cell counter (Hialeah, FL) was not affected by E2 or P in these ASMC (data not shown). The number of ASMC initially plated was adjusted so that the cell densities at the end of each experiment were similar. ASMC were plated and maintained for 24 h in phenol red-free medium supplemented with 20% charcoal-stripped FBS. The medium was replaced for 24 h with 0.1% charcoal-stripped FBS containing vehicle (0.001% ethanol, control wells), physiological concentrations of E2 (0.1 and 1 nmol/l), P (1 and 10 nmol/l), and/or the ER antagonist ICI-182780 (ICI; Tocris, Ballwin, MO) and the PR inhibitor RU-486 (RU; Sigma). Confluent cell layers were harvested for RNA and/or protein analysis or collagen measurements. Supernatants were collected for measurement of MMP activity and collagen. All experiments (duplicate wells for each condition) were performed in triplicate.

**Isolation of mRNA and real-time PCR.** Total RNA was extracted from the aortic arch or confluent cell layers using Tri-Reagent (3, 26). Primers and probes were purchased from PerkinElmer Applied Biosystems (Foster City, CA) and used as specified by the manufacturer’s protocol. The sequences of the primers used for mouse ERα and ERβ were as published (26). Sequences for PR, MMP-2, MMP-9, type IV collagen, and transforming growth factor (TGF)-β primers and probes are listed in Table 1. Real-time RT-PCR reactions were performed using the TaqMan One Step RT-PCR Master Mix reagent kit and the ABI Prism 7700 sequence detection system (PerkinElmer Applied Biosystems) in a total volume of 50 μl of reaction mixture. A TaqMan ribosomal probe RNA control reagent kit was used to detect the 18S ribosomal RNA gene, which represented an endogenous control. Each sample was normalized to the 18S transcript content as previously described (26). The standard curves for each molecule were generated using serial dilutions (0.001–100 ng) of mRNA from mouse uterus. PCR assays were conducted in duplicate for each sample (100 ng total RNA/well). Data are expressed as percentage of C3H and represent the means ± SE of tissue extracts from three mice for each group and for ASMC of three independent experiments in duplicate for each group.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Primer Sequences and TaqMan Probe</th>
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<tbody>
<tr>
<td>PR</td>
<td>GGGACTGCAGAAGCTTCTCTTT</td>
</tr>
<tr>
<td></td>
<td>GAGTTTTATGCAAATGCAGCAT</td>
</tr>
<tr>
<td></td>
<td>FAM-AATAGTTATGCTGGCTTTGTCGAAAT</td>
</tr>
<tr>
<td></td>
<td>TAMRA</td>
</tr>
<tr>
<td>MMP-2</td>
<td>CCGCAGTGAAGCCTGTTGTTTTA</td>
</tr>
<tr>
<td></td>
<td>TGGAGGGCGGAAACGGAAACT</td>
</tr>
<tr>
<td>MMP-9</td>
<td>FAM-TGGCAATGCTGATGGAGACGGCCTA-TAMRA</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>ACTGCGATCTTTCGCCGCTTC</td>
</tr>
<tr>
<td>α1-Type IV collagen</td>
<td>GCCATGCCGCGAGCATCCGAGCA</td>
</tr>
<tr>
<td></td>
<td>TAMRA</td>
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For each molecule, the sequence in the 1st line is the forward primer and the sequence in the 2nd line the reverse primer; the 3rd line represents the TaqMan probe sequence. All sequences are shown 5’ to 3’. ASMC, aortic smooth muscle cells; PR, progesterone receptor; MMP, matrix metalloproteinase; TGF-β, transforming growth factor-β; FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine; VIC, dye (Applied Biosystems).
Western blots. Protein expression was examined by Western blot as described (25, 26). Antibodies against ERα (H-184, MC-20), ERβ (Y-19), and PR (C-20, H-190) and their respective blocking peptides were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Briefly, confluent cell layers were washed once in PBS, and protein was extracted with a lysis buffer. Equal amounts of protein lysates or immunoprecipitates from each experimental condition were run on a 10% PAGE. Experiments were performed in the presence of ERα and ERβ human recombinant peptides or protein extracted from mouse uterus as positive controls, and the specificity of the signal was demonstrated by incubating blots with an excess of the corresponding specific immunizing peptide. Densitometry was performed using ImageJ 1.17 (National Institutes of Health, Bethesda, MD) to determine relative amounts of protein.

Three independent experiments were performed in duplicate. Results are expressed as a percentage of C3H-ASMC.

RESULTS

**ER subtypes-α and -β expression was higher in aorta and ASMC from B6 than from C3H mice.** ER mRNA levels were higher in aortic tissue isolated from B6 than from C3H mice (ERα = 2.0 and ERβ = 14.5-fold higher, *P < 0.05*). The mRNA levels of both ER subtypes were also higher in B6-ASMC than in C3H-ASMC. Because the results were identical in the second set of cultured ASMC, the data were pooled and represent the average of the two sets of cultured ASMC. ERα and ERβ mRNA levels in B6-ASMC were 2.1- and 15.9-fold higher than in C3H-ASMC (*P < 0.001* and *P < 0.05*, respectively; Fig. 1A). ERα mRNA levels were 16- to 20-fold higher than ERβ mRNA levels in both tissue and ASMC from C3H and B6 mice.

ERα and ERβ protein expression was higher (1.7- and 1.5-fold, *P < 0.001* and *P < 0.05*, respectively) in B6-ASMC than in C3H-ASMC (Fig. 1B). PR expression was higher in aorta and ASMC from B6 than from C3H mice. PR mRNA levels were higher in aortic tissue isolated from B6 than from C3H mice (*P < 0.01; Fig. 2A). Similarly, PR mRNA levels in B6-ASMC (2 independent sets of cultured ASMC) were higher than those found in C3H-ASMC (*P < 0.05; Fig. 2A). In both aortic tissue and ASMC of C3H and B6 mice, PR mRNA levels were comparable to the levels of ERβ mRNA expression.

PR protein expression was 1.3-fold higher in B6-ASMC compared with C3H-ASMC (*P < 0.05; Fig. 2B).

ER transcriptional activity was higher in B6-ASMC than in C3H-ASMC. We assessed the transcriptional activity of endogenous ER by transfecting ASMC isolated from B6 and C3H mice with a luciferase-based reporter gene. The luciferase expression from this reporter plasmid was under the transcriptional control of four consecutive, synthetic ERE (the plasmid was kindly provided by Dr. D. Shapiro, University of Illinois, Urbana, IL). A physiological concentration of 1 nM E2 induced a 2.2-fold increase in luciferase activity in B6-ASMC (*P < 0.01; Fig. 3). There was no luciferase response in C3H-ASMC at this E2 concentration. However, the low transfection efficacy that we observed could decrease the overall sensitivity of this assay in both C3H and B6 cell lines. Nevertheless, these transfection studies unequivocally demonstrated that the ER expressed in atherosclerosis-susceptible B6-ASMC are transcriptionally active.

**Type IV collagen was higher in B6-ASMC than in C3H-ASMC, and estrogens decreased type IV collagen synthesis only in B6-ASMC.** Type IV collagen mRNA levels were lower in aortic tissue and ASMC isolated from C3H than in B6 mice (*P < 0.05* and *P < 0.001*, respectively; Fig. 4A). Type IV collagen protein was higher in B6-ASMC than in C3H-ASMC (*P < 0.001; Fig. 4B). The amounts of type IV collagen in the cell layers were higher than those measured in the supernatants for both C3H- and B6-ASMC (*P < 0.001; Fig. 4B).

E2 decreased type IV collagen synthesis in B6-ASMC but not in C3H-ASMC (*P < 0.05; Fig. 4C). The same
results were obtained in the cell layers. Namely, E2 decreased type IV collagen synthesis in B6-ASMC (61.6 ± 4.9 and 71.9 ± 4.9% of control, P < 0.05 for 0.1 and 1 nmol/l of E2, respectively) but not in C3H-ASMC cell layers. The ER antagonist ICI abolished the E2-induced decrease in type IV collagen synthesis in B6-ASMC (1 μmol/l, 110.5 ± 7.5 and 114.3 ± 8.2% of control in the cell layer and supernatant, respectively), whereas ICI alone had no effect.

In contrast, P did not change type IV collagen synthesis in either cell layer or supernatant of C3H or B6-ASMC (Table 2).

**Type I collagen was higher in B6-ASMC than in C3H-ASMC, and estrogens decreased type I collagen synthesis only in B6-ASMC.** Type I collagen was higher in the cell layers than in the supernatants for both C3H- and B6-ASMC (P < 0.05; Fig. 5A). Type I collagen was higher in B6-ASMC than in C3H-ASMC cell layers (P < 0.05; Fig. 5A). Type I collagen protein expression was higher than type IV collagen in both C3H- and B6-ASMC cell layers and supernatants (P < 0.001; Figs. 4 and 5).

E2 decreased type I collagen synthesis in B6-ASMC supernatants (P < 0.05), whereas there was no change in C3H-ASMC (Fig. 5B). E2 also decreased type I collagen synthesis in B6-ASMC cell layers (71.8 ± 13.2 and 76.1 ± 4.7% of control, P < 0.05 for 0.1 and 1 nmol/l of E2, respectively). The ER antagonist ICI abolished the E2-induced decrease in type I collagen synthesis in B6-ASMC (1 μmol/l, 110.8 ± 9.5 and 124.6 ± 8.0% of control for cell layer and supernatant, respectively), whereas ICI alone had no effect. P did not change type I collagen synthesis in either C3H- or B6-ASMC cell layers or supernatants (Table 2).
Thus the synthesis of type IV and type I collagen was higher in ASMC from atherosclerosis-susceptible than from atherosclerosis-resistant mice. Estrogens decreased type IV and type I collagen synthesis only in ASMC from atherosclerosis-susceptible mice. However, progestins did not affect type IV or type I collagen synthesis in either B6-ASMC or C3H-ASMC.

Baseline MMP-2 and response to estrogens and progestins differed in B6-ASMC and C3H-ASMC mice. MMP-2 mRNA expression was similar in aortic tissue from B6 and C3H mice. MMP-2 mRNA levels were 3.6-fold higher \((P < 0.01)\) in B6-ASMC than in C3H-ASMC (Fig. 6A). Figure 6B shows a representative zymogram of MMP-2 activity in C3H- and B6-ASMC. B6-ASMC had a twofold higher MMP-2 activity than C3H-ASMC \((P < 0.001)\).

E2 (1 nmol/l) increased MMP-2 activity \((131.5 \pm 9.8\% \text{ of control, } P < 0.001)\) in C3H-ASMC but decreased it in B6-ASMC \((P < 0.001; \text{ Fig. 6C})\). The ER antagonist ICI abolished this effect \((1 \mu \text{mol/l, } 88.2 \pm 8.3\% \text{ of control})\), and ICI alone had no effect. In contrast, P decreased MMP-2 activity in B6-ASMC but not in C3H-ASMC \((P < 0.001; \text{ Fig. 6D})\). The PR antagonist RU abolished this effect \((10 \mu \text{mol/l, } 89.2 \pm 2.4\% \text{ of control})\), whereas RU alone did not change MMP-2 activity.

Baseline MMP-9 activity and response to estrogens and progestins differed in B6-ASMC and C3H-ASMC mice. MMP-9 mRNA expression was similar in aortic tissue and ASMC isolated from C3H and B6 mice (Fig. 7A). Figure 7B shows a representative zymogram of MMP-9 activity in C3H- and B6-ASMC. MMP-9 activity was 1.6-fold higher in B6-ASMC than in C3H-ASMC \((P < 0.001; \text{ Fig. 7B})\).

E2 (1 nmol/l) decreased MMP-9 \((77.8 \pm 5.9\% \text{ of control, } P < 0.05)\) activity in C3H-ASMC but not in B6-ASMC (Fig. 7C). The ER antagonist ICI abolished this effect \((1 \mu \text{mol/l, } 121.2 \pm 9.6\% \text{ of control})\), but ICI alone had no effect. In contrast, P decreased MMP-9 activity in B6-ASMC \((P < 0.05 \text{ and } P < 0.001 \text{ for 1 and } 10 \mu \text{mol/l, respectively})\) but not in C3H-ASMC (Fig. 7D). This effect was abolished by the PR antagonist RU \((10 \mu \text{mol/l, } 82.3 \pm 6.7\% \text{ of control})\), and RU alone had no effect.

Thus ASMC isolated from atherosclerosis-susceptible mice express higher MMP-2 and MMP-9 levels than...
ASMC isolated from atherosclerosis-resistant mice. Progestins decreased MMP activity only in ASMC isolated from atherosclerosis-susceptible mice. Estrogens increased MMP-2 activity but decreased MMP-9 activity in the atherosclerosis-resistant mice, whereas MMP-2 activity was decreased in the atherosclerosis-susceptible mice.

**DISCUSSION**

B6 and C3H mice differ in their susceptibility to diet-induced atherosclerosis (21). Because cholesterol levels are higher in sclerosis-resistant C3H than in sclerosis-susceptible B6 mice, the cell type-specific effects and responses to atherogenic factors may play a fundamental role in the development of atherosclerotic lesions in these mouse models. We studied the aortic arch because this is the region where B6 mice develop atherosclerotic lesions. We also isolated ASMC from the aortic arch, because they contribute to arterial intimal thickening and the formation of atherosclerotic lesions.

The degree of susceptibility or resistance to developing atherosclerosis in the B6 and C3H strains is determined by at least eight genes (21). The interplay of these genetic factors may influence the direct effects of estrogen and progestin on ASMC during the development and progression of arterial vessel disease. They may also determine whether certain individuals are unresponsive or may respond in an unfavorable fashion to sex hormones. Such a phenomenon could be responsible for the increased event rate during the first year and the overall null effect of the first randomized prospective trial studying the outcome of HRT in the secondary prevention of cardiovascular disease in postmenopausal women (HERS). In addition, no cardiovascular benefit was observed in the subsequent open-label observational follow-up HERS II. Similarly, the rate of women experiencing CHD events was increased by 29% for postmenopausal women taking estrogen plus progestin relative to placebo in the primary prevention WHI study (31).

**Table 2. Progestins did not affect type IV and type I collagen synthesis in C3H- and B6-ASMC**

<table>
<thead>
<tr>
<th>[P], nmol/l</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>0</th>
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<th>10</th>
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<tbody>
<tr>
<td><strong>Type IV collagen, %control</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C3H</td>
<td></td>
<td></td>
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<tr>
<td>Cell layer</td>
<td>100.0 ± 3.8</td>
<td>101.7 ± 9.9</td>
<td>98.67 ± 3.9</td>
<td>100.0 ± 11.3</td>
<td>98.46 ± 11.4</td>
<td>97.1 ± 14.3</td>
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<td>Supernatant</td>
<td>100.0 ± 3.4</td>
<td>104.7 ± 7.0</td>
<td>97.8 ± 4.8</td>
<td>100.0 ± 8.6</td>
<td>102.4 ± 12.1</td>
<td>96.6 ± 10.2</td>
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<tr>
<td>Cell layer</td>
<td>100.0 ± 4.6</td>
<td>96.7 ± 9.6</td>
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<td>100.0 ± 8.3</td>
<td>87.3 ± 9.7</td>
<td>81.0 ± 13.4</td>
</tr>
<tr>
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<td>99.9 ± 6.6</td>
<td>85.0 ± 4.3</td>
<td>100.0 ± 8.4</td>
<td>90.1 ± 6.7</td>
<td>78.1 ± 14.3</td>
</tr>
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</table>

C3H/HeJ (C3H) and C57B1/6J (B6) ASMC were treated with progesterone (P; 0, 1, or 10 nmol/l) for 24 h. Cell layers and supernatants were collected, and type IV and type I collagen were measured by ELISA. Data are expressed as % control (vehicle-treated cells). Shown are means ± SE of 3 independent experiments performed in duplicate wells. There was no statistical significance compared with control.
2.4-fold increase of gene activity in vascular smooth muscle cells (VSMC) by use of a supraphysiological dose of E2 ($10^{-7}$ M). However, the low sensitivity of this assay might also explain the apparent lack of luciferase activity in the sclerosis-resistant C3H-ASMC. Therefore, the results of our experiments may not truly reflect the inability of ER in atherosclerosis-resistant C3H-ASMC to transcriptionally activate an ERE but rather the low copy number of transfected ERE-driven reporter genes. On the other hand, differences in coac-
tivator or corepressor levels could further contribute to this phenomenon.

Our findings of increased sex hormone receptor levels in the ASMC of atherosclerosis-susceptible mice contrast with a previous study in humans (18), which suggested that ER expression was lower in the coronary arteries of women with CHD. Thus the results of our study in mice do not support the hypothesis that atherosclerotic lesions develop only because of low ER expression and/or decreased intrinsic ER function in ASMC. However, we cannot entirely exclude the possibility that exposure to a high-fat diet would down-regulate the expression of sex hormone receptors and/or alter ER and/or PR function, a potential atherosclerosis-promoting phenomenon that we have not studied.

However, in B6 mice, activation of ER and PR does not appear to prevent atherosclerotic lesion formation, as these mice develop atherosclerosis on a high-fat diet during the reproductive phase of their life, when sex steroids are abundant (22). This is supported by the fact that the frequency of regular estrous cycles in B6 mice is three times higher than that of C3H mice (20). The higher frequency of regular estrous cycle with E2 and P levels ranging from 47.3 ± 2.1 to 66.0 ± 3.2 pg/ml (0.174 to 0.242 nM) and 1.2 ± 0.5 to 18.4 ± 3.6 ng/ml (3.8 to 58.5 nM), respectively, may in fact translate into higher mean circulating sex hormone levels in B6 compared with C3H mice.

Transflecting an ERE-containing reporter gene into an ER-expressing cell type provides a means to assess the transcriptional activity of endogenous ER in the context of an artificial and simple promoter. However, because we have not assessed the presence or the different expression of different coactivators and/or corepressors between B6-ASMC and C3H-ASMC, we cannot exclude the possibility that this would limit ERE activation in this particular context. Furthermore, although these experiments are useful to determine the transcriptional functionality of steroid hormone receptors, they do not necessarily provide information on the transcriptional response of specific endogeneous target genes following stimulation by sex hormones. Thus, to determine the specific role of ER and/or PR activation on molecules that are involved in arterial wall remodeling, migration of ASMC, and the stability of atherosclerotic lesion, we examined the effects of E2 and P on type IV collagen, type I collagen expression by ASMC might play a pivotal role in lesion instability. A similar phenomenon in humans could explain the higher incidence of myocardial events in women with CHD receiving HRT (HERS, HERS II) (6, 10).

In summary, our study shows that the levels of ER and PR in ASMC isolated from the aortic arch are not associated with the propensity to develop atherosclerotic lesions in C3H and B6 mice. Furthermore, E2 and P differentially regulate collagen synthesis and MMP activity in ASMC from atherosclerosis-susceptible and atherosclerosis-resistant mice. These findings emphasize the importance of the genetic background in determining the direct and different
ent responses of ASM to estrogens and progestins, a phenomenon that could account for the contradictory results obtained with HRT in postmenopausal women.

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DISCLOSURES

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