Endothelial dysfunction of resistance vessels in female apolipoprotein E-deficient mice

Maine S Cola1, Agata L Gava1, Silvana S Meyrelles1 and Elisardo C Vasquez*1,2

Abstract
Background: The effects of hypercholesterolemia on vasomotricity in apolipoprotein E-deficient (ApoE) mice, a murine model of spontaneous atherosclerosis, are still unclear. The studies were mostly performed in conductance vessels from male mice fed a high-fat diet. In the present study, we evaluated the endothelial function of resistance vessels from normal C57BL/6 (C57) and hypercholesterolemic (ApoE) female mice in both normal and ovariectomized conditions.

Methods: Twenty week-old C57 and ApoE mice underwent ovariectomy or sham surgery and were studied 30 days later. The vascular reactivities to norepinephrine (NE, 10^-9 to 2 × 10^-3 mol/L), acetylcholine (ACh) and sodium nitroprusside (SNP) (10^-10 to 10^-3 mol/L) were evaluated in the isolated mesenteric arteriolar bed through dose-response curves.

Results: ACh-induced relaxation was significantly reduced (P < 0.05) in ApoE compared with C57 animals, as indicated by both the maximal response (37 ± 4% vs. 72 ± 1%) and the LogEC50 (-5.67 ± 0.18 vs. -6.23 ± 0.09 mol/L). Ovariectomy caused a significant impairment in ACh-induced relaxation in the C57 group (maximal response: 61 ± 4%) but did not worsen the deficient state of relaxation in ApoE animals (maximal response: 39 ± 5%). SNP-induced vasorelaxation and NE-induced vasoconstriction were similar in ApoE and C57 female mice.

Conclusion: These data show an impairment of endothelial function in the resistance vessels of spontaneously atherosclerotic (ApoE-deficient) female mice compared with normal (C57) female mice. The endothelial dysfunction in hypercholesterolemic animals was so marked that ovariectomy, which impaired endothelial function in C57 mice, did not cause additional vascular damage in ApoE-deficient mice.

Background
Atherosclerosis is a slow, progressive and multifactorial disease that results from interactions between genetic and environmental factors [1]. The murine model that lacks the gene encoding apolipoprotein E (ApoE) and spontaneously develops hypercholesterolemia and atherosclerotic lesions similar to those found in human beings [2,3] has greatly contributed to the understanding of this disease. In this animal, on a chow diet, as early as 10 weeks of age, monocyte adhesions are observed, followed by intermediate lesions containing foam and smooth muscle cells at 15 weeks and sequentially by fibrous plaques at 20 weeks of age [4]. Although endothelial dysfunction has been considered one of the early steps in atherosclerosis [5], its occurrence in ApoE-deficient mice is still controversial. Studies in conductance vessels of male and female ApoE-deficient mice have shown both normal endothelial function [6,7] and endothelial dysfunction [8-11]. On the other hand, studies in resistance vessels from ApoE-deficient mice have shown normal endothelial function in males [12,13] and endothelial dysfunction in females [14]. It is well established that estrogens exert several direct effects on the vessel wall [15-18] and that post-menopausal women [19] are known to present an elevated risk of cardiovascular events, which can be reduced by exogenous administration of 17β-estradiol [20,21]. In accordance with this view, it has been shown that the loss of female hormones leads to the impairment of endothelial function in rats [22-24], but this was not yet investigated in mice.

* Correspondence: evasquez@pq.cnpq.br
1 Laboratory of Transgenes and Cardiovascular Control, Physiological Sciences Graduate Program, Health Sciences Center, Federal University of Espirito Santo, Vitória, ES, Brazil
Full list of author information is available at the end of the article

© 2010 Cola et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
The importance of studying resistance vessels is based on the fact that arterioles act as control conduits through which blood is released into tissue microcirculation. Considering that the responsiveness of resistance vessels is still poorly understood in the mouse and that there are few studies in atherosclerotic female mice, the present study was designed to evaluate endothelial function in the isolated mesenteric arteriolar bed of C57BL/6 and ApoE-deficient mice under both normal and ovariectomy conditions.

**Methods**

The experiments were performed using 20-24-week-old female C57BL/6 (C57) and ApoE mice obtained from the animal facilities of the Health Sciences Center at the Federal University of Espirito Santo. The animals were housed according to institutional guidelines for animal research and fed a normal diet. The procedures were previously approved by the institutional Ethics Committee for Use of Animals (CEUA-EMESCAM, protocol #002/2009) and were conducted in accordance with the international guidelines for care and use of laboratory animals.

At 20 weeks of age, C57 and ApoE mice underwent ovariectomy, as previously described [22]. Thirty days after ovariectomy, the animals were anesthetized (ketamine/xylazine, 91.0/9.1 mg/kg, i.p.) and blood samples were collected for determination of 17β-estradiol and total cholesterol levels. 17β-estradiol levels were determined using chemiluminescence assays (Marcos Daniel Laboratory, Vitoria, Brazil) and total cholesterol levels were measured colorimetrically using a commercial kit (Bioclin, Sao Paulo, Brazil). After blood sample collection, the superior mesenteric artery was cannulated. The mesenteric arteriolar bed was then transferred to a 37°C water container and perfused at a constant rate (2 mL/min) with oxygenated (95% O2 - 5% CO2 mixture) physiological salt solution (in mmol/L: 130 NaCl, 4.7 KCl, 1.6 CaCl2·2H2O, 1.18 KH2PO4, 4.7 MgSO4·7H2O, 14.9 NaHCO3, 0.026 EDTA and 11.1 glucose) using a peristaltic pump (Harvard Apparatus, Holliston, MA, USA). Perfusion pressure was monitored via a T-tube inserted between the pump and the inflow cannula and connected to a pressure transducer and a data acquisition system (BioPac Systems, Goleta, CA, USA). The dose-response curves to norepinephrine (NE, Sigma Chemical Co. Saint Luis, MO, USA, 10⁻⁹ to 2 × 10⁻³ mol/L), acetylcholine (ACh, Sigma, 10⁻¹⁰ to 10⁻³ mol/L) and sodium nitroprusside (SNP, Sigma, 10⁻¹⁰ to 10⁻³ mol/L) were then performed in the isolated mesenteric arteriolar bed. The vascular responses were evaluated as changes in the perfusion pressure and the vasodilator responses to ACh and SNP were calculated as percentages of the contractions induced by NE (5 × 10⁻⁶ mol/L).

All data are expressed as means ± SEM. Statistical analysis was performed with 1- or 2-way ANOVA followed by Tukey's post hoc test for multiple comparisons. The level of significance was set at P < 0.05.

**Results**

Body weight, uterus weight, uterus weight/body weight ratio, plasma 17β-estradiol and cholesterol levels are summarized in Table 1. Thirty days after ovariectomy, body weight was not altered; however, uterus weight was significantly reduced in ovariectomized groups, with no differences between C57 and ApoE animals. Consequently, the uterus weight/body weight ratio was diminished in about 80% in the ovariectomized groups when compared with the respective sham groups. The success of ovariectomy was also confirmed by plasma 17β-estradiol levels, which were significantly lower in ovariectomized groups (2-fold, P < 0.01) in comparison with sham animals, also without differences between C57 and ApoE mice. As expected, plasma cholesterol levels were signific-

### Table 1: Body and uterus weight and plasma 17β-estradiol and cholesterol levels in C57 and ApoE-deficient mice groups

| Parameters                  | Sham   | C57    | ApoE   |
|-----------------------------|--------|--------|--------|
|                             | 25.9 ± 0.5 (22) | 26.8 ± 0.6 (22) | 26.3 ± 0.6 (12) | 25.5 ± 0.7 (12) |
| Body weight (g)             | 30.3 ± 1.8 (22) | 5.4 ± 0.7* (22) | 36.2 ± 1.6 (12) | 5.9 ± 1.3† (12) |
| Uterus weight (mg)          | 1.18 ± 0.10 (22) | 0.20 ± 0.02* (22) | 1.38 ± 0.07 (12) | 0.23 ± 0.05† (12) |
| Uterus weight/body weight (mg/g) | 128 ± 8 (14) | 61 ± 4* (13) | 152 ± 12 (11) | 71 ± 3† (9) |
| Plasma 17β-estradiol (pmol/L) | 1.66 ± 0.13 (16) | 1.42 ± 0.11 (13) | 10.77 ± 1.02* (11) | 9.64 ± 0.88* (9) |

Data are reported as means ± SEM of the number of animals indicated in parentheses. OVX: ovariectomized. *P < 0.05 vs C57 Sham; †P < 0.05 vs ApoE Sham (ANOVA).
Significantly higher (6.5-fold) in ApoE than in C57 mice. Hypercholesterolemia levels of ApoE mice were not affected by ovariectomy.

The endothelium-dependent vasodilatation of the isolated mesenteric arteriolar bed in response to ACh is shown in Figure 1. The vascular responsiveness to ACh was significantly reduced in ApoE animals compared with C57 animals, as indicated by both the maximal response (37 ± 4% vs. 72 ± 1%, P < 0.01) and the LogEC50 (-5.67 ± 0.18 vs. -6.23 ± 0.09 mol/L, P < 0.05) (panel A).

Ovariectomy caused a significant impairment in the ACh-induced relaxation in the C57 group (panel C) but did not worsen the deficient state of relaxation in ApoE animals (panel D). Even after ovariectomy, ACh-induced relaxation still was significantly diminished in ApoE animals compared with C57 animals (maximal response: 39 ± 5% vs. 61 ± 4%, P < 0.01, panel B).

The endothelium-independent vascular smooth muscle relaxation in ApoE mice produced by the nitric oxide donor SNP was statistically similar the relaxation in C57 mice in both intact and ovariectomized animals. The vascular constriction response to NE was also not affected by the hypercholesterolemic condition of ApoE animals or by ovariectomy.

Discussion
One of the hallmarks of atherosclerosis in humans is the impairment of endothelial function [5]. The ApoE-deficient mouse is considered one of the most relevant models for atherosclerosis because this animal develops

---

**Figure 1** Endothelium-dependent relaxations produced by acetylcholine in mesenteric arteries of C57 and ApoE mice 30 days after ovariectomy (OVX) or sham surgery (Sham). Responses are expressed as the percentage of relaxations relative to the NE-induced precontractions. A: comparison between C57 Sham and ApoE Sham. B: comparison between C57 OVX and ApoE OVX. C and D: effects of ovariectomy on acetylcholine-induced vasorelaxation in C57 and ApoE mice, respectively. Data are reported as means ± SEM (n = 8 to 10 per group). *P < 0.05, **P < 0.01 vs respective control (ANOVA).
spontaneous arterial lesions [2,3]. Resistance vessels of male ApoE mice generally present normal endothelial function on a normal chow diet [12,13] and endothelial dysfunction is only observed in high-fat diet conditions [25]. In contrast, female ApoE mice, even on a normal diet, show endothelial dysfunction in aorta and cerebral resistance vessels [9,14]. In the present study, we show that the isolated mesenteric arteriolar bed of female ApoE-deficient mice fed a normal diet also presents endothelial dysfunction, as indicated by the marked decrease in ACh-induced vasorelaxation. The impairment of vasorelaxation does not appear to involve the vascular smooth muscle because the endothelium-independent vasorelaxation to SNP was preserved in the female ApoE-deficient mouse. On the other hand, in the present study we observed a normal NE-induced vasoconstriction in resistance vessels of female ApoE-deficient mice, corroborating the finding by others showing preserved responses to NE in aorta rings of female ApoE-deficient mice under both normal and high-fat diets [8]. In this model, the normal vascular responsiveness to NE seems to be related only to females because in previous study it was found an exacerbated NE-induced vasoconstriction in male mice [12].

Gender is a potent risk factor for cardiovascular diseases [26]. In contrast with females, males have an increased prevalence of atherosclerotic-based diseases, such as ischemic heart disease, stroke and aortic aneurysms [27,28]. In the present study, we investigated resistance vessel responsiveness in both normal and spontaneously atherosclerotic female mice. In agreement with others [29-31], mice subjected to the removal of ovaries presented a smaller uterus weight in comparison with non-castrated mice. In the present study, we observed that ovariectomy led to a significant reduction in ACh-induced vasorelaxation in female C57 mice. Although impairment of endothelial function has been observed in conductance vessels of castrated female mice, dysfunction of resistance vessels of C57 animals had not yet been reported. Based on other studies, this finding could be explained by the fact that estrogen has direct beneficial effects on endothelial function by augmenting prostacyclin and NO synthesis [17,32-34].

Contrasting with males [12,13], we observed that female ApoE-deficient mice on a normal chow diet show endothelial dysfunction of resistance vessels. Similar results have been observed by others in the aorta and cerebral arterioles of female ApoE-deficient mice [9,14]. These observations corroborate the finding that female ApoE-deficient mice present a higher vascular lesion area than male mice [35], which appears to be related to direct effects of estrogen on the specific immune activation on female mice. In the present study, the ovariectomy of female ApoE-deficient mice did not cause additional damage to the endothelial function of resistance vessels, probably because the animals already presented marked endothelial dysfunction. This finding seems to be related only to the ApoE-deficient mouse because it is well known that in other models of cardiovascular diseases estrogen deficiency leads to endothelial dysfunction [17,22]. Similar to C57 female mice, we did not observe any effect of ovariectomy on the NE-induced vasoconstriction in resistance vessels of female ApoE-deficient mice, corroborating the finding that female 17β-estrogen receptor knockout mice did not present any change in NE-induced vasoconstriction [36].

In conclusion, the current findings demonstrated an impairment of endothelial function in resistance vessels of spontaneously atherosclerotic (ApoE-deficient) female mice compared with normal (C57) female mice. The endothelial dysfunction was so marked that ovariectomy, which impaired the endothelial function in C57, did not cause additional damage in ApoE-deficient mice. Further studies will be required to elucidate the mechanisms involved in the endothelial dysfunction in these animals. We speculate that increased superoxide anion production and reduced activity of endothelial nitric oxide synthase could contribute to the endothelial dysfunction in female ApoE-deficient mice.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MSC carried out the animal experiments, analysis of the data, statistics and drafted the manuscript. ALG carried out the ovariectomy surgery and participated in manuscript preparation. SSM participated in the design and co-supervision of the study and in the critical revision of the manuscript. ECV conceived the study, participated in its design and supervision and in the critical revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This research was supported by the National Council for the Development of Science and Technology (CNPq, Ref. 302113/2008-8 Grant), State Agency for the Development of Science and Technology (FAPES, Ref PRONEX), and Funds for Science and Technology of the City of Vitoria (FACITEC).

Author Details
1Laboratory of Transigens and Cardiovascular Control, Physiological Sciences Graduate Program, Health Sciences Center, Federal University of Espirito Santo, Vitoria, ES, Brazil and 2Laboratory of Cardiovascular Pathophysiologys, Research Center, Esmscam College of Health Sciences, Vitoria, ES, Brazil

Received: 5 April 2010 Accepted: 19 May 2010
Published: 19 May 2010

References
1. Ross R: Atherosclerosis: an inflammatory disease. N Engl J Med 1999, 340:115-126.
2. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N: Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 1992, 89:4471-4475.
3. Plump AS, Smith JD, Hayek T, Aalto-Seidala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL: Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 1992, 71:343-353.
4. Meir KS, Leitersdorf E: Atherosclerosis in the apolipoprotein E-deficient mouse: a decade of progress. Arterioscler Thromb Vasc Biol 2004, 24:1096-1104.
5. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993, 362:801-806.
6. Gervais M, Pons S, Nicotelli A, Cosson C, Giudicelli J-F, Richer C: Fluvastatin prevents renal dysfunction and vascular NO deficit in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2003, 23:183-189.
7. Villeneuve N, Fortuno A, Fournier N, Breugnot C, Jacquemin C, Lüscher TF: Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc Natl Acad Sci USA 1994, 91:5212-5216.
8. Deckert V, Lizard G, Duverger N, Ascaso A, Palleau V, Emmanuel F, Moisant M, Gambert P, Lallemant C, Lagrost L: Persistence of the nitric oxide pathway in the aorta of hypercholesterolemic apolipoprotein E-deficient mice. J Vasc Res 2003, 40:87-96.
9. Niebauer J, Maxell AJ, Lin PS, Tsao PS, Kosek J, Bernstein D, Cooke JP: Impaired aerobic performance in hypercholesterolemic mice: partial reversal by exercise training. Am J Physiol Heart Circ Physiol 1999, 276:H1346-H1354.
10. d’Uscio LV, Smith LA, Katusic ZS: Hypercholesterolemia impairs endothelium-dependent relaxations in common carotid arteries of apolipoprotein E-deficient mice. Stroke 2001, 32:2658-2664.
11. Li J, Kim JR, Scott RS, Kowalski LR: Protection against endothelial dysfunction induced by oxidized low-density lipoproteins in isolated mouse aorta; a comparison with apolipoprotein E-deficient mice. Eur J Pharmacol 2001, 424:141-149.
12. Arruda RMP, Peotta VA, Meyrellas SS, Vasquez EC: Evaluation of vascular function in apolipoprotein E knockout mice with angiogenesis-dependent renovascular hypertension. Hypertension 2005, 46:932-936.
13. Wolfe SE, de Wit C: Intact endothelium-dependent dilation and conducted responses in resistance vessels of hypercholesterolemic mice in vivo. J Vasc Res 2005, 42:475-482.
14. Kitayama J, Faraci FM, Lentz SR, Heistad DD: Cerebral vascular dysfunction during hypercholesterolemia. Stroke 2007, 38:2136-2141.
15. Mendelsohn ME: Mechanisms of estrogen action in the cardiovascular system. J Steroid Biochem Mol Biol 2000, 74:337-343.
16. Amal J-F, Bayard F: Vasculoprotective effects of oestrogens. Clin Exp Pharmacol Physiol 2001, 28:1032-1034.
17. Tostes RC, Nigro D, Fortes ZB, Carvalho MHC: Effects of estrogen on the vascular system. Braz J Med Biol Res 2003, 36:1143-1158.
18. Maturana MA, Ingoglia MC, Spritzer PM: Menopause, estrogens, and endothelial dysfunction: current concepts. Clinics 2007, 62:77-86.
19. Carr MC, Kim KH, Zambon A, Mitchell ES, Woods NF, Casazza CP, Purnell JQ, Hokanson JE, Brunzell JD, Schwartz RS: Changes in LDL density across the menopausal transition. J Invest Med 2000, 48:245-250.
20. Chae CU, Ridker PM, Manson JE: Postmenopausal hormone replacement therapy and cardiovascular disease. Thromb Haemost 1997, 78:770-780.
21. Qiao X, McConnell KR, Khaliq RA: Sex steroids and vascular responses in hypertension and aging. Endocr Rev 2008, 29:468-484.
22. Dantas APV, Scivoletto R, Fortes ZB, Nigro D, Carvalho MHC: Influence of female sex hormones on endothelium-derived vasoconstrictor prostaglandin generation in microvessels of spontaneously hypertensive rats. Hypertension 1999, 34:914-919.
23. Molini K, Khemaprech S, Patumraj S, Siririyakul P: Preventive mechanism of goiters in coronary endothelial dysfunction in ovariectomized rats: an isolated arrested heart model. Clin Hemorheol Microcirc 2004, 31:59-66.
24. Stice JP, Eisnerich JP, Knowlton AA: Role of aging versus the loss of estrogens in the reduction in vascular function in female rats. Endocrinology 2005, 150:212-219.
25. d’Uscio LV, Barton M, Shaw S, Luscher TF: Chronic ETα receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. Cardiovasc Res 2002, 53:497-495.
26. Lenwand LA: Sex is a potent modifier of the cardiovascular system. J Clin Invest 2003, 112:302-307.
27. Rodin MB, Davgles MS, Wong GC, Liu K, Garside DB, Greenland P, Stamler J: Middle age cardiovascular risk factors and abdominal aortic aneurysm in older age. Hypertension 2003, 42:561-568.
28. Blanchard JF, Armenian HK, Friessen P: Risk factors for abdominal aortic aneurysm: results of a case-control study. Am J Epidemiol 2000, 151:575-583.
29. Marsh MM, Walker VR, Curtiss LK, Banka CL: Protection against atherosclerosis by estrogen is independent of plasma cholesterol levels in LDL receptor-deficient mice. J Lipid Res 1997, 40:893-900.
30. Hodgin JB, Krege JT, Reddick RL, Korach KS, Smithies O, Maeda N: Estrogen receptor α is a major mediator of 17β-estradiol’s atheroprotective effects on lesion size in ApoE-/- mice. J Clin Invest 2001, 107:333-340.
31. Henriches TA, Huang J, D’Souza SS, Daugherty A, Cassis LA: Orchiectomy, but not ovariectomy, regulates angiotensin II-induced vascular diseases in apolipoprotein E-deficient mice. Endocrinology 2004, 145:3866-3872.
32. Weiner CP, L’opez I, Baylis SA, Knowles RG, Charles IG, Moncada S: Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc Natl Acad Sci USA 1994, 91:5212-5216.
33. Jun SS, Chen Z, Pace MC, Shaul PW: Estrogen upregulates cyclooxygenase-1 gene expression in ovine fetal pulmonary artery endothelium. J Clin Invest 1998, 102:176-183.
34. Ospina JA, Krause DN, Durkies SP: 17β-Estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. Stroke 2002, 33:600-605.
35. Caligiuri G, Nicotelli A, Zhou X, Tomberg J, Hansson GK: Effects of sex and age on atherosclerosis and autoimmunity in apoE-/- mice. Atherosclerosis 1999, 145:301-308.
36. Douglas GS, Cruz MN, Pinston L, Gustafsson J-A, Kubilieka K: Functional characterization and sex differences in small mesenteric arteries of the estrogen receptor-β knockout mouse. Am J Physiol Regul Integr Comp Physiol 2009, 294:112:120.